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2022

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Integrative Medicine 2022,

20–21 July 2022

Integrative Medicine : Trusted Care



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President's Welcome Message



On behalf of Mae Fah Luang University, I would like to welcome all participants to Mae Fah Luang University and to the 2nd International Conference in Integrative Medicine 2022: Integrative Medicine: Trusted Care.

The year, 2022, is very special to Mae Fah Luang University (MFU) marking the 24th anniversary of our establishment. Since 1998, MFU has committed itself to follow Her Royal Highness Princess Srinagarindra's aspirations in nurturing nature and developing people for national and regional development.

For 24 years, MFU has put its effort into being a leading university in the Greater Mekong Subregion (GMS) and expanding to ASEAN with international recognition. We have produced quality graduates and impactful research that responds to society's needs and creates impacts in many sectors of the country. Moreover, we have offered several high-quality academic programs in the field of integrative medicine including Applied Thai Traditional Medicine, Traditional Chinese Medicine, and Physical Therapy. Our programs are designed to meet the international standards to ensure that our knowledge and innovations will be able to share with the world.

From the number of participants gathered here today and the number of papers submitted for the conference, I am confident that this conference will contribute to the future collaboration in all fields of integrative medicine and to the network of healthcare professionals from different parts of the world.

In this regard, I also would like to thank you all of the conference participants for your contributions to this event.

Finally, I sincerely hope that all the participants will benefit from the academic and practical experience sharing at this conference and wish this conference a complete success.

Sincerely Yours,

Assoc. Prof. Dr. Chayaporn Wattanasiri

President of Mae Fah Luang University



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Conference Chair's Welcome Message



Integrative Medicine is not a new name in the field of medical work. It is a term that describes the use of other forms of medicinal practice in combination with modern medicine to treat diseases, prevent diseases and also improve or rehabilitate patients back to good health. Nowadays, both healthcare practitioners and patients are getting more interested in the efficacy and what integrative medicine can offer. Herbal medicine and Traditional medicine are practices that everyone is familiar with and are willing to try it out. Although they are well known in many parts of the world, many aspects of them still lack a better understanding from a scientific aspect.

For these reasons the School of Integrative Medicine, Mae Fah Luang University, Thailand, is committed to preserve and improve these fields of practice in both producing graduates with good competency and high-quality research. The International Conference on Integrative Medicine 2022 or ICIM is the 2nd ICIM that the school has held. We have seen a greater perception from the general public along with many success cases, our patients have called us “trusted care”. I believe that this conference will be a great platform for healthcare professionals and scientists in this field of work to come and connect with each other. Moreover, participants who participate in this conference would get a clearer view and get to update on the new techniques and knowledge in the field of integrative medicine. So, I hope all participants will receive new knowledge, make new friends, and hope this conference succeeds in all of its objectives.

Dr.Sulakkana Noiprasert, M.D.

Chairperson of the International Conference on Integrative Medicine 2022

Introduction

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MAE FAH LUANG UNIVERSITY



Mae Fah Luang University (MFU) was established as an autonomous public university, under the Royal Charter, in 1998, with generous support from the Royal Thai Government. The University was established to meet the needs of people in the north of Thailand, and to commemorate the gracious contributions of the King's Mother, Her Royal Highness Princess Srinagarindra, lovingly known to her subjects as "Mae Fah Luang." From its inaugural class of 64 students in 1998, MFU has become Thailand's fastest growing post-secondary institution with an enrollment of just under 15,000 students. After little over a decade of operation, MFU is already well known for its high-quality teaching, research, and service to the people of the north, as well as Thailand.

MFU's campus consists of a large complex of modern, state-of-the-art buildings, spanning more than 800 hectares. The campus is located in a spectacular setting of mountains and trees, an environment conducive to teaching and learning in a clean, inviting setting. As both a regional and national university, MFU provides high-quality education services and resources for the neighboring countries of the Greater Mekhong Sub-region (GMS), and reaches out to all of Southeast Asia.

MFU's philosophy is to "restore forests and develop people" and declared has goals of "New Different Better Together". Our vision is to a leading university in ASEAN with international recognition. We will produce quality human resources and develop excellence in academics, research, and innovations for the society under disruptions to achieve the Sustainable Development Goals in accordance with Thailand's development.





School of Integrative Medicine



The School of Integrative Medicine separated from the School of Health Science on the 1st of June 2019 and established the 15th School of Mae Fah Luang University under the name of “School of Integrative Medicine”. The school aims to provide academic and professional excellence in the field of integrated medicine with international standards and to promote health care through traditional medical care and a wide range of therapies.

At the present, the school of Integrative Medicine offers 3 Bachelor’s programs, Bachelor of Physical Therapy, Applied Thai Traditional Medicine, and Traditional Chinese Medicine. Moreover, the School is in the planning stage to offer the additional postgraduate program in Master of Integrative Medicine and Master of Physical Therapy.





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Organizing committee

- 1. Dr. Sulukkana Noiprasert (Chairperson)**
Ph.D. (Acupuncture and Massage)
Department of Traditional Chinese Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 2. Assoc. Prof. Dr. Pattanasin Areeudowmong**
Ph.D. (Human Movement Sciences)
Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 3. Dr. Natthawut Yodsuwan**
Ph.D. (Biotechnology)
Department of Biological Science, School of Science, Mae Fah Luang University, Chiang Rai, Thailand
- 4. Asst. Prof. Dr. Tawatchai Apidechkul**
Ph.D. (Public Health Sciences)
Center of Excellence for the Hill tribe Health Research, Mae Fah Luang University, Chiang Rai, Thailand and Department of Public Health, School of Health Science, Mae Fah Luang University, Chiang Rai, Thailand
- 5. Assoc. Prof. Dr. Vitsarut Buttagat**
Ph.D. (Human Movement Sciences)
Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 6. Dr. Pravaree Phuneerub**
Ph.D. (Public Health Sciences)
Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 7. Dr. Chakree Wattanasiri**
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Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 8. Dr. Parichart Hongsing**
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- 9. Dr. Aunyachulee Ganopichayagrai**
Ph.D. (Public Health Sciences)
Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand



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Scientific committee

- 1. Assoc. Prof. Dr. Vitsarut Buttagat**
Ph.D. (Human Movement Sciences)
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- 2. Assoc. Prof. Dr. Pattanasin Areeudowmong**
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- 3. Asst. Prof. Dr. Rawiwan Charoensup**
Ph.D. (Public Health Sciences)
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- 4. Dr. Pravaree Phuneerub**
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- 5. Dr. Chakree Wattanasiri**
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- 6. Dr. Parichart Hongsing**
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- 7. Dr. Aunyachulee Ganogpichayagrai**
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Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 8. Dr. Sujittra Klauyhomthong**
Ph.D. (Human Movement Sciences)
Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand
- 9. Dr. Chatchada Satalangka**
Ph.D. (Neuroscience)
Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand



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10. Dr. Thidarat Duangyod

Ph.D. (Public Health Sciences)

Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

11. Dr. Kitiyawadee Srisim

Ph.D. (Human Movement Sciences)

Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

12. Dr. Nakornsub Lawcharoen

Ph.D. (Acupuncture and Tuina)

Department of Traditional Chinese Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

13. Dr. Patcharawan Suwannarat

Ph.D. (Human Movement Sciences)

Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

14. Dr. Chin Jia Wei

M.Sc. (Endocrinology)

Department of Traditional Chinese Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

15. Prof. Wu Yun Chuan

Ph.D (Acupuncture and Moxibustion)

Nanjing University of Chinese Medicine, China

16. Prof. Wei Li Xin

M.Sc. (Acupuncture)

China Academy of Chinese Medical Sciences, China

17. Prof. Lian Ai Hua

Ph.D. (Traditional Chinese Medicine Pharmacology and Toxicology)

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

18. Prof. Dr. Dirk Moller

Ph.D. (Dipl. Sportwiss., Physiotherapy)

Faculty of Business Management and Social Sciences (Physical Therapy) Osnabrück University of Applied Sciences, Germany

19. Dr. Christopher Zaslowski

Ph.D. (Biological Sciences)

College of Traditional Chinese medicine, University of Technology, Sydney, Australia



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20. Dr. Rebekka Zirbel

Ph.D. (Biological Sciences)

The European Society for Applied Immunology, Germany

21. Dr. Wen Te Chung

Ph.D., Institute of Pharmaceutical Sciences

Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Chinese Medicine, China Medical University, Taiwan

22. Dr. Supannikar Yingyongsaksri

Ph.D. (Physical Therapy)

Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand

23. Dr. Donlaya Promkeaw

Ph.D. (Human Movement Sciences)

Department of Physical Therapy, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand



Virtual Conference at Mae Fah Luang University: Schedule (Thailand time, UTC +7)

20th July 2022

08.30 - 09.00	Registration
09.00 – 09.25	Opening Ceremony Overview of ICIM 2022 by Dr. Sulukkana Noiprasert Chairperson of ICIM 2022 Opening Speech by Assoc. Prof. Dr. Chayaporn Wattanasiri President of Mae Fah Luang University
09.25 – 09.30	Taking picture session
09.30 – 10.30	Plenary Lecture 1 Topic: Application of Liquid Biopsy, Life Cell Imaging and Plasma Proteins Electrophoresis in Natural Medicine: Identify Causes and Assess the Treatment Outcome of Siamois® polyphenols Keynote Speaker: Prof. Dr. Samlee Mankhetkorn
10.30 – 10.45	Coffee Break
10.45 – 11.15	Plenary Lecture 2 Topic: Somatic Pain and the Role for Acupuncture - an Australian Example of Strategic Research Keynote Speaker: Assoc. Prof. Dr. Christopher Zaslowski
11.15 – 12.00	Oral Presentation
12.00 – 13.00	Lunch Break
13.00 – 13.30	Plenary Lecture 3 Topic: Acupuncture in Primary care Keynote Speaker: Prof. Dr. Wei Li Xin



13.30 – 13.40

Plenary Lecture 4

Topic: Risk Assessment, Medication Safety and Scientific Supervision of Traditional Chinese Medicines Containing Aristolochic Acids—Different Types of Aristolochic Acids Have Different Toxicities, and the Detection and Control of Aristolochic Acids I and II Are Critical

Keynote Speaker: Prof. Dr. Liang Ai Hua

13.40 – 16.00

Oral Presentation

16.00 – 17.15

e-Poster Presentation

21st July 2022

08.00 - 08.30

Registration

08.30 – 10.30

Plenary Lecture 5

Topic: Nuad Thai (Thai massage) for Relieving Office Syndrome in the Digital Generation

Keynote Speaker: Assoc. Prof. Dr. Wichai Euangpinichpong

10.30 – 10.45

Coffee Break

10.45 – 12.00

Oral Presentation

12.00 – 13.00

Lunch Break

13.00 – 14.30

Plenary Lecture 6

Topic: Can Good Gut Health Prevent Diseases

Keynote Speaker: Dr. Johannes Wessolly

14.30 – 15.30

Plenary Lecture 7

Topic: The role of Thai-based Herbal Medicine in COVID-19 Management

Keynote Speaker: Asst. Prof. Dr. Rawiwan Charoensup

15.30 – 16.30

Oral Presentation

16.30 – 17.00

Award Announcement and Closing Ceremony



Schedule (Thailand time, UTC +7) for Oral presentation 20 th July 2022 Virtual Conference at Mae Fah Luang University					
Section	Room	Time	OCS no.	Presenter's name	Title
1	1-chinese language	11.15-11.30	6	Miss. Wachiraporn Padungkiettiwong	Analysis of the clinical therapeutic effect of Professor Wang Yue on 42 cases of Sjogren's syndrome overlaps with Hashimoto's thyroiditis
		11.30-11.45	84	Mr. Jia Wei Chin	Traditional Chinese Medicine in treating depression
		11.45-12.00	28	Dr. Nakornsub Lawcharoen	The history and development of TCM ointment therapy
	2-chinese language	11.15-11.30	61	Miss. Raksuda Taniguchi	Clinical research on the treatment of hyperthyroidism with Chaihu Guizhi Longu Muli Decoction
		11.30-11.45	56	Mr. Pitchayut Chambumrung	Research advance of lumbar disc herniation treated by special type of acupuncture
	3-Eng language	11.15-11.30	78	Dr. Ampha Pumpho	Impact of scoliosis on gait characteristics: a case study of a child with spastic diplegic cerebral palsy
		11.30-11.45	92	Miss. Supapon Kaewsanmung	Immediate effect of Thai massage on calf muscle flexibility and postural sway in overweight children: pilot study
		11.45-12.00	49	Dr. Kitiyawadee Srisim	Relationship between balance ability and ankle function in diabetes mellitus patients with peripheral neuropathy (DMPN)
	2	1-Eng language	13.55-14.10	10	Miss. Panitha Boonma
14.10-14.25			97	Miss. Vassana charoonsrisawad	The Effect of Court-Type Thai Traditional Massage on Delayed-Onset Muscle Soreness in The Biceps Brachii Muscle After Resistance Exercise



Schedule (Thailand time, UTC +7) for Oral presentation
20th July 2022 Virtual Conference at Mae Fah Luang University

Section	Room	Time	OCS no.	Presenter's name	Title	
		14.25-14.40	36	Mr. Pornnarez Thaweekhotr	Surface Anatomical Positioning of the 2nd and 3rd Shoulder Signal Points of Court-type Thai Massage in Male Humans	
		14.40-14.55	79	Miss. Tanyarath Tapcompill	Effect of aromatic medicine (YA HOM TEP PRA CHIT) on patient with hypertension	
		14.55-15.10	88	Mr. Sirikool klumkool	The Effectiveness of Thai Massage on the range of motion and muscular strength of the arm in collegiate basketball players, Mae Fah Luang University	
		15.10-15.25	89	Mrs. Panada Ramphaiboon	Effect of Court-Type Thai Traditional Massage Versus <i>Cassia alata</i> Linn. Treatment on Chronic Constipation: A Randomized Controlled Trial	
		15.25-15.40	94	Miss. Parichart Hongsing	A systematic review of randomized controlled trial on efficacy of <i>Centella Asiatica</i> for wound healing	
		15.40-15.55	99	Mr. Thanach Kanokthet	Effect of a pain management Combined with Mahajak oil medicine program in elderly with osteoarthritis: A Case study of Thay Nam Health Promoting Hospital, Pho Thale District, Phichit Province	
	2-chinese language		13.55-14.10	5	Dr. Song Yufan	Application of tonifying kidney, invigorating Qi and promoting blood circulation in common gynecological diseases
			14.10-14.25	66	Miss. Wang Yuanwang	Research on Operation Method of Tuina Treatment of Constipation in Children Based on Data Mining Technology
			14.25-14.40	103	Miss. YU jia	Clinical Observation of Magui Wenbi Granules in the Treatment of Rheumatoid Arthritis and Its Effect on IL-23 / IL-17 Axis
			14.40-14.55	100	Mr. Sin Khee Ho	Discussion on the Application of Pharmacological Theory of Traditional Chinese Medicine in the Treatment of the Covid 19 Cases
			14.55-15.10	107	Mr. Zhu Xinyu	Study of Acupuncture Improving Oocytes Quality of Poor Ovarian Responders in IVF



Schedule (Thailand time, UTC +7) for Oral presentation
20th July 2022 Virtual Conference at Mae Fah Luang University

Section	Room	Time	OCS no.	Presenter's name	Title
3-Eng language		13.55-14.10	3	Prof. Dr. Dirk Möller	The effect of muscle fatigue on activation patterns of the biceps femoris and semitendinosus muscles in soccer
		14.10-14.25	83	Miss. Chanyawat Rueangsri	Content validity of Thai version screening tool for cervical spine instability
		14.25-14.40	25	Miss. Benjamaporn Hancharoenkul	Relationship between predisposing factors and risk of work-related musculoskeletal strain among poultry slaughterhouse workers – A cross sectional study
		14.40-14.55	27	Miss. Wilawan Chaiut	Relationship between the 2- and 6- Minute Walk Test in Healthy Children
		14.55-15.10	24	Miss. Yadanuch Boonyaratana	Measuring movements 24 hours per day in older persons living in a nursing home - a pilot study
		15.10-15.25	21	Prof. Dr. Nikolaus Ballenberger	Occurrence of musculoskeletal health complaints and corresponding risk factors among music students and non-music students – results from a prospective cohort study
		15.25-15.40	65	Miss. Nichapa Khumpaneid	Effects of modified-OTAGO on body composition in older adults during COVID-19 outbreak: A preliminary study
		15.40-15.55	101	Dr. Petcharat Keawdaungdee	Prevalence and Associated Factors of Forward Head Posture among Physical Therapy Students



Schedule (Thailand time, UTC +7) for Oral presentation
21st July 2022 Virtual Conference at Mae Fah Luang University

Section	Room	Time	OCS no.	Presenter's name	Title
1	1-Eng language	10.45-11.00	64	Dr. Nawatpong Chairat	A pilot study of chemical composition and major compounds of conservation plant: a case study of Ma-kwan from Doi Phu Kha, Nan Province
		11.00-11.15	16	Miss. Ajchamon Thammachai	Knowledge, Awareness, and Practice Regarding Pesticide Exposure and Health Symptoms among Farmers
		11.15-11.30	110	Dr. Sujitra Klauyhomthong	The effect of combined the pursed lips and lower costal breathing exercise and chest mobilization program on lung volume and dyspnea in patients with chronic obstructive pulmonary disease after acute exacerbations (AECOPD) and hospitalization
		11.30-11.45	51	Mr. Ritichai Pimpa	Development of Alcohol Behavioral Assessment Tool for Hill Tribe Adolescents
		11.45-12.00	105	Dr. Supannikar Yingyongsaksri	Correlation between perceived severity of COVID-19 and self-protective behavior during COVID-19 pandemic in Chiang Rai smokers
	2-Eng language	10.45-11.00	8	Mr. Jirapak Ruttanapattanakul	Proliferation and Survival Enhancing Effect of Pinocembrin on Immortal Human Epidermal Cells
		11.00-11.15	26	Mr. Phatarawat Thaklaewphan	The anti-cancer effects of oxyresveratrol on ovarian cancer cell lines via negative regulation of AKT activation status
		11.15-11.30	98	Miss. Kanyaphat Apiwongsrichai	Effects of extraction solvent on red pigment, phenolic compounds, and antioxidant capacity of red mold rice extracts
		11.30-11.45	87	Miss. Khin Thapyay Phyu	The Effectiveness of 5% White Tea (<i>Camellia sinensis</i>) Cream for Periorbital Wrinkle Reduction
		11.45-12.00	96	Miss. Nichaphat Wisetchonlathan	Metadata and the fatty acids content in Thai shrimp paste
	3-Eng language	10.45-11.00	72	Dr. Patcharawan Suwannarat	Discriminative Ability of the 7th Cervical Vertebral Wall Distance for hyperkyphosis measurement



Schedule (Thailand time, UTC +7) for Oral presentation 21 st July 2022 Virtual Conference at Mae Fah Luang University					
Section	Room	Time	OCS no.	Presenter's name	Title
		11.00-11.15	55	Dr. Chatchada Sotalangka	Comparison of executive function in male puberty and andropause
		11.15-11.30	54	Miss. Janya Chuadthong	The immediate effects of star excursion balance training on balance and walking ability in healthy elderly people: A randomized controlled trial
		11.30-11.45	69	Dr. Ekalak Sitthipornvorakul	The effect of Kinesio taping on back muscle endurance, lower back flexibility and balance in sedentary young adult
		11.45-12.00	104	Miss. Watjanarat Panwong	Comparison Physiological Effect and Comfortable Sensation between Wearing Surgical Face Mask and Muslin Face Mask during Exercise in Healthy Subject: A Pilot Study
2	1-Eng language	15.30-15.45	39	Mr. Napongpakorn Chumsri	Prevalence and unit cost of abnormalities detected in abdominal sonography among soldiers
		15.45-16.00	47	Dr. Amornsak Poom	Factors Affecting to Breast Cancer Screening for Breast Self-examination among Women in Phitsanuloke Province
		16.00-16.15	41	Miss. Supaporn Intatham	Effect of Thai herbal recipes on the immune system in streptozotocin-induced diabetic retired breeder mice
	2-Eng language	15.30-15.45	68	Miss. Amisa Laprom	Molecular characterization of the full-length bipartite begomovirus causing pepper yellow leaf curl disease, a common cause of crop damage in northeast and central Thailand
		15.45-16.00	34	Dr. Aunyachulee Ganogpichayagrai	Antimicrobial activities of <i>Streblus asper</i> leaves extract in Thailand
	3-chinese language	15.30-15.45	23	Shihui Zheng	Therapy of strengthening spleen and Nourishing Kidney Massage in the Treatment of Global Developmental Delay
		15.45-16.00	22	Aihua Liang	Risk assessment, safe medication and scientific supervision of traditional Chinese medicines containing aristolochic acids



Schedule (Thailand time, UTC +7) for Oral presentation
21st July 2022 Virtual Conference at Mae Fah Luang University

Section	Room	Time	OCS no.	Presenter's name	Title
		16.00-16.15	48	Zhangsimei	Study on the mechanism of exercise therapy in promoting neural stem cell mobilization after stroke
		16.15-16.30	95	Zhang Shouyao	Comprehensive therapy based on pediatric tuina in the treatment of Idiopathic Thrombocytopenic Purpura: A case report
		16.30-16.45	106	Zhou Li	Effects of electroacupuncture on FUNdC1-LC3 signaling pathway in rats cerebral ischemia-reperfusion injury
		16.45-17.00	50	Miss. Sinee Tantasatityanon	Case Report: Treating Atopic Dermatitis with Si-Wu-Xiao-Feng Decoction in combination with Topical Huang Qin Cream
		17.00-17.15	108	Yin Mengxin	Research progress on osteogenic differentiation of bone marrow mesenchymal stem cells induced by Traditional Chinese medicine monomer



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Schedule (Thailand time, UTC +7) for E-poster

Number	OCS No.	Track	Time	Presenter's name	Title
1	20	APM	16.15-16.18	Dr. Warangkhana Klajing	Therapeutic Efficacy of Knee Acupuncture added Sanyinjiao (SP6) point for Pain Reduction in Knee Osteoarthritis Patients
2	58	APM	16.18-16.21	Miss. Pornfah Ananpaisarn	Data analysis of acupoints selection in acupuncture treatment for post-stroke depression based on Traditional Chinese Medicine Inheritance Support System (TCMISS)
3	13	CMD	16.21-16.24	Assoc Prof. Somrudee Saiyudthong	Linalool exerted a neuroprotective activity against corticosterone-induced apoptosis of PC12 cells
4	18	EPH	16.24-16.27	Miss. Kanyarat Pengngummuang	Anti-inflammatory effect of folk remedies for hemorrhoids treatment
5	37	EPH	16.27-16.30	Dr. Yothin Teethaisong	Anti-biofilm potential of Boesenbergia rotunda L. extract and synergy with cloxacillin on biofilms of β -lactam-resistant Staphylococcus aureus
6	93	EPH	16.30-16.33	Miss. Namphung Thongta	The protective effect of Ayurved Siriraj Wattana on chronic cerebral hypoperfusion induced by bilateral common carotid artery ligation
7	53	HMN	16.33-16.36	Miss. Nongnapat Leelasithorn	The Effects of Orange, Sweet Basil, and Dok Mok Essential Oils Inhalation on Emotional State and Autonomic Nervous System
8	74	IM	16.36-16.39	Asst.Prof.Dr. Narunan Wuttisin	Psychological effects of nail color
9	30	IM	16.39-16.42	Miss. Natchanun Phunthanateerakul	Role of intravenous glutathione and high-dose vitamin C in adult acute lymphoblastic leukemia adjunctive therapy
10	85	IM	16.42-16.45	Mr. Pattarapol Techasuwan	A comparative study for clinical efficacy and safety between a combination of fractional radiofrequency and microneedling with fractional radiofrequency alone in the treatment of striae distensae
11	73	IM	16.45-16.48	Miss. Thanchanok Muangman	The protective effects of marine microalgae extract on skin cells-induced sun ray



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Schedule (Thailand time, UTC +7) for E-poster

Number	OCS No.	Track	Time	Presenter's name	Title
12	80	IM	16.48-16.51	Dr. Haruthai Petviset	Development of Problem Solving by Project-Based Learning in Physical Therapy Student, Mae Fah Lung University
13	62	PHP	16.51-16.54	Mr. Chiramet Auranwiwat	Ultrasonic assisted extraction enhanced total phenolic and antioxidant activities from <i>Aegle marmelos</i> (L.) Corr. extract
14	52	RMT	16.54-16.57	Miss. Ploypailin Namkorn	The effect of external-focused attention using the prototype model of SCF innovation (Speedy Colors Fitting) on postural control in healthy young adults
15	7	TMD	16.57-17.00	Mr. Sitthichock vadphimai	A cross-sectional survey of Traditional Chinese Medicine constitution in Thailand's Type 2 diabetic population
16	102	TMD	17.00-17.03	Dr. Witayapan Nantitanon	Antiglycation, α -glucosidase, and α -amylase inhibitory activity of guava leaf hydrosol
17	86	TMD	17.03-17.06	Mr. Jia Wei Chin	Effectiveness of Huangqi Guizhi Wuwu decoction on adult diabetic neuropathic: a systematic review and meta-analysis
18	49	IM	17.06-17.09	Miss. Peeranan Pattanamongkol	Research progress in the prevention and treatment of osteoporosis with exercise therapy
19	111	TMD	17.09-17-12	Miss. Chuleekorn Kwanchainon	The herbal medicine of Vernonia cinerea. tea Comparing the Effectiveness of Herbal Medicine with Placebo to anti - cigarette smoking. U-Thong Hospital, Suphanburi, THAILAND

Keynote speaker abstract

ICIM 2022



Application of Cell-based Liquid Biopsy, Life Cell Imaging and Plasma Proteins Electrophoresis in Natural Medicine: Identify Causes and Assess the Treatment Outcome of Siamois® Polyphenols



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ABSTRACT

This is the first demonstration of bridging the gap of natural medicine and the interlocking of cells as body's basic building blocks and microenvironments. It is the reason why the natural medicine deals with the concepts that build on the extraordinary ability of body to heal itself. Today's such kind of the interlocking of cells and microenvironments can clearly show by using cell-based liquid biopsy for *ex-vivo* monitoring the behavior of cells, while the proposed mechanisms were studied by gel electrophoresis of plasma protein. Moreover, the correlation of cell behavior in *ex-vivo* and in human body conditions can clearly demonstrated by using life cell and infrared thermal imaging, respectively. The concepts of cell-based liquid biopsy, secret-omics in plasma and infrared thermal imaging was applied in order to identify and assess treatment outcome of Siamois® polyphenols was realized in the SCL medical clinic in cooperation with the cancer research and treatment center as evidence based in natural medicine for almost 10 years. Where 100,000 investigations were performed for 9,000 participants. In this talk, some testimonial studies of both symptomatic and asymptomatic on health status will be discussed. We thus propose here for the first time that the application of these methodologies can answer to (a) how to visualize and determine the perfect conditions and (b) how to adjust or break down the limiting steps that influence on the repair and regeneration processes of tissues.



Somatic Pain and the Role for Acupuncture - an Australian Example of Strategic Research



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ABSTRACT

Support for acupuncture - NICE report

- The NICE (National Institute for Health and Care Excellence) recommendations for chronic pain were published in April 2021) confirming the role acupuncture has as one of three evidence supported therapies for the treatment of chronic pain.
- The report, titled “Chronic pain (primary and secondary) in over 16s: assessment of all chronic pain and management of chronic primary pain” reported that many studies (27 in total) showed that acupuncture reduced pain and improved quality of life in the short term (up to 3 months) compared with usual care or sham acupuncture.
- The committee agreed that “there was a large evidence base showing acupuncture to be clinically effective in the short term (3 months).”

Series of studies for acute (experimental) and chronic pain studies (RCTs)

- Several studies have been conducted at the University of Technology (UTS) Acupuncture research group over the last 20 years looking at the modulation of pain using pressure pain threshold as a pain challenger.
- LI 4 (Hegu) (study 1, 2003)
- LI 4 (Hegu) with LI 11 (Quchi) (study 2, 2008)
- LI 4 with electroacupuncture (study 3, unpublished)
- LI 11 (Quchi) and LI 10 (Shousanli) for tennis elbow (study 4, 2020) - two pilot studies (Australia and China), systematic review, published protocol.



PPT studies (studies 1-3) - acute experimental pain

- Used a pain challenger (Pressure Pain Threshold) called an algometer which measured pressure
- Measured PPT at sites across the body in healthy humans
- Timeline of measurement and intervention procedures

Study 1 - Four needling interventions ^[1]

1. Deep needling of LI4 with manipulation LI4m+ D (TCM needling)
2. Deep needling of a non-acupoint with manipulation NAPm+ D (sham control)
3. Deep needling of a non-acupoint (NAP) without manipulation NAPm- D (sham control)
4. Inactive Laser (placebo)

Summary

Within treatment (significant increase from baseline)

- LI4m⁺ D: significant increase at 10 sites
- NAPm⁺ D: significant increase at 5 sites
- NAPm⁻ D: significant increase at 1 site
- Inactive Laser: no significant increases observed
- Needling LI4 with manipulation produced mean increases that were statistically significantly greater than those for the other interventions with one exception: needling the nonacupoint with manipulation was as effective as needling LI4 with manipulation at one measurement site only.

Study 2- LI 4 (hegu) and LI 11 (Qu chi) ^[2]

The same manual acupuncture techniques were applied to four interventions of:

- 1) LI4(hegu) unilaterally;
- 2) LI4 bilaterally (hegu x 2);
- 3) LI 11 (quchi) unilaterally;
- 4) LI4 in conjunction with LI11, both unilaterally (LI4 + LI 11).

Following all four interventions, statistically significant increases in mean PPT were observed.

These occurred at

- nine measurement sites following the LI4 intervention either unilaterally or bilaterally;
- six measurement sites for LI11 intervention;
- five measurement sites following the combined LI11 and LI4 intervention.

These increases were significantly greater for the bilateral LI4 intervention than the unilateral LI4 intervention at only two measurement sites.

Study 3 (unpublished)

Aim - to compare the effects of manual acupuncture, electroacupuncture and TENS to acupoint LI4 on regional pressure pain thresholds.

It was a randomized and dual-blinded (subject and assessor) study involving 24 healthy volunteers.

The three interventions were

- 1) TENS to LI4, TENS
- 2) Electroacupuncture to LI4 and
- 3) Manual Acupuncture to LI4.

Outcome measures - Pressure pain threshold (PPT) was measured before and after each intervention at ten sites (seven acupoints and three nonacupoint) across the body. In addition, subjects rated on a visual analogue scale (VAS) their subjective levels of pain, intervention sensation and tension



experienced during, and anxiety prior to, the intervention.

- All three interventions elicited significant statistical increases in regional PPT.
- The effects were generalized across the body. The TENS intervention was the least effective and electroacupuncture was the most effective.
- The effects on regional PPT following manual acupuncture were consistent with previous studies.

Tennis Elbow Acupuncture-International Study-China, Hong Kong, Australia, and Italy (TEA-IS-CHAI) ^[3]

An example of an international collaborative RCT project with five partners Chinese Medicine Team, UTS

- Changchun University of TCM, China -School of Chinese Medicine, Hong Kong Baptist University, Hong Kong
- Istituto Paracelso, Italy
- World Federation of Acupuncture and Moxibustion Societies (WFAS), China
- Utilised several acupoints we had evaluated previously especially LI 11 (Quchi)

Lateral elbow pain (tennis elbow) is a painful condition that is associated with the degeneration in the area of common extensors tendon of the forearm.

Tennis elbow study

- This study was an international, prospective, multi-center, randomized, controlled, clinical trial to evaluate the efficacy of acupuncture compared to sham laser in the treatment of Lateral Elbow Pain (tennis elbow).
- The study used a parallel and stratified design. Subjects from 18 to

80 years with unilateral chronic LEP (minimum three months) were recruited at four centers in Australia, China, Hong Kong and Italy (n=96).

- Intervention - The treatment group received manual acupuncture at acupoints LI 10 and LI 11 on the affected side whereas the control group received sham-laser acupuncture at the same acupoints.
- Outcomes - The primary endpoint was the disabilities of the arm, shoulder, and hand (DASH) questionnaire score at the three-week post-treatment follow-up visit. A visual analogue scale (VAS) was also used as a secondary outcome to measure pain at rest and during movement.
- Both the DASH score and the pain VAS on two occasions (at rest and during motion) showed a significant change over time indicating acupuncture as a potential treatment for LEP due to tendinosis.
- Acupuncture was shown to be efficacious in treating the pain and function of lateral elbow tendinosis. Both the DASH score and VAS pain both at rest and during motion showed a significant change over time indicating acupuncture as a potential treatment for LEP due to tendinosis.

Conclusion

- Value in conducting some preliminary studies prior to the controlled trial for tennis elbow.
- Allowed the research team to evaluate several acupoints including LI 11 and LI 4 before commencing a larger clinical trial.

Gave the research team familiarity by undertaking earlier studies using PPT as an outcome measure in the RCT



References

- [1] Zaslowski, C., Cobbin, D. Lidums. E. and Petocz, P. (2003). A randomised single blind study of the effects of site and magnitude of needle manipulation associated with acupuncture on pain pressure threshold. *Complementary Therapies in Medicine*, 7, 1, p.11-21.
- [2] Li, W-H., Cobbin, D. and Zaslowski, C. (2008) A comparison of effects on regional pressure pain threshold produced by deep needling of LI4 and LI11, individually and in combination. *Complementary Therapies in Medicine*, 14 (5):527-36.
- [3] Gadau etal (2020) A multi-center international study of acupuncture for lateral elbow pain - Results of a randomized controlled trial. *European Journal of Pain*, 24; 8. p. 1458-1470



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2nd International Conference on Integrative Medicine 2022
Integrative Medicine : Trusted Care
Mae Fah Luang University, Chiang Rai, Thailand

Acupuncture in Primary care



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ABSTRACT

To introduce acupuncture & moxibustion therapy, including definition of acupuncture, features of acupuncture, and clinical application of acupuncture for postpartum hypogalactia, xerophthalmia and polycystic ovary syndrome, which we've studied in recent years.



Risk Assessment, Medication Safety and Scientific Supervision of Traditional Chinese Medicines Containing Aristolochic Acids—Different Types of Aristolochic Acids Have Different Toxicities, and the Detection and Control of Aristolochic Acids I and II Are Critical



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ABSTRACT

The safety of traditional Chinese medicines containing aristolochic acid has attracted much attention in China and other countries, which is a significant difficulty for their clinical application and drug supervision. There have been 178 types of aristolochic acid analogues (AAAs) reported. According to their structure, AAAs can be divided into aristolochic acids (AAs) and aristolactams (ALs). Different types of AAAs have different toxicities. For example, AA-I shows strong nephrotoxicity and carcinogenicity; the toxicity of AA-II is lower than that of AA-I; and AA-IVa and AA-Ia are not considered to have notable nephrotoxicity or carcinogenicity. The types and contents of AAAs in traditional Chinese medicines from the Aristolochiaceae family vary widely. Some varieties, such as *Asarum sieboldii* and *Fibraurea recisa* Pierre, consist mainly of AAAs without obvious toxicity (such as AA-IVa). Compound preparations containing traditional Chinese medicines from Aristolochiaceae could greatly reduce the retention of AAAs due to their low proportions and preparation procedure. The content of AA-I in some compound preparations is very low or undetectable. Therefore, one author proposed that “Different types of AAAs have different toxicities, and not all AAAs show nephrotoxicity and carcinogenicity. The toxicity of traditional Chinese medicines containing AAAs should not be generalized, and we should focus on controlling the contents of AA-I and AA-II”. Herein, it is suggested that we should use traditional Chinese medicines containing AAAs rationally; strengthen research, analysis and toxicological studies of the types of AAAs and their contents; establish standard limits for AA-I and AA-II; and provide a scientific basis for rational clinical applications and drug supervision.



Nuad Thai (Thai massage) for Relieving Office Syndrome in the Digital Generation



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ABSTRACT

Office syndrome is a group of symptoms such as neck; shoulder, and back pain resulting from sitting or working in the same position for long periods. Sometimes it has been recognized as repetitive strain injury. It always affects musculoskeletal tissues including muscles, tendons, fascia, and ligaments as they are vulnerable to repeated injury when the persons are restricted in the same posture. The digital age enhanced by pandemic diseases such as Covid19 could induce more prevalent office syndrome because people tend to work hard, physically, and mentally, online and are restricted in sitting posture. Once the body tissues could not tolerate the demanding work, they could have inflamed and degeneration. Nuad Thai or Thai massage has been recognized both in Thailand and worldwide for promoting relaxation of body and mind. Physiologically, Nuad Thai has been found to improve local blood circulation, tissue perfusion, body posture, and parasympathetic activities. Therefore, it may have a major role in the alleviation of musculoskeletal tissue injuries and pain due to office syndrome. Possible mechanisms could be based on changing posture and applying deep massage to the whole body to provide blood circulation to the affected tissues and facilitate repairing processes. Moreover, it could reduce muscle and soft tissue pain resulting from releasing the tension of the tightened tissues and facilitate mental relaxation. Some simple techniques of Nuad Thai to the neck, shoulder and back will be demonstrated. These include thumb pressure along the meridian lines, and Thai Yoga stretches. It is expected that the audience could get some ideas and may practice along to deliver some beneficial effects of Nuad Thai for those who suffer from office syndrome.



Can Good Gut Health Prevent Diseases?



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ABSTRACT

A healthy intestine is the root of all health (Hippocrates 460 to 370 B.C.)

We associate a large number of diseases with the intestine, not only direct intestinal diseases but also diseases of the metabolism, immune system and psyche as well as allergic diseases.

Microbiome

All of these diseases are closely linked to the intestinal microbiome, which consists of about 38 trillion bacteria. They affect the health of various organs, for example, liver, lungs, immune system, even the brain.

Brain

Low diversity of the microbiome may have a critical impact in the development of Alzheimer's disease. Here, the production of butyrate by certain intestinal bacteria is particularly noteworthy, because butyrate is crucial for the function of the microglia, the intrinsic immune system of the brain.

Liver

Almost all of the blood from the intestine is directed to the liver via the portal vein. Toxic metabolites of pathogenic intestinal bacteria can thus induce a non-alcoholic fatty liver. In addition, the enteric autonomic nervous system (ENS) becomes stressed and causes sleep disturbances.

Immune system

The influence of the intestinal microbiome on the function of the immune system is crucial. 70-80% of immune cells are located in the intestinal barrier, where they maintain a constant exchange of information with intestinal bacteria and are trained in this way. Pathogenic bacteria elicit false immune responses, promoting the development of a leaky gut, a type of silent inflammation that has negative effects on the entire organism.



Infancy

The development of the intestinal microbiome begins in the womb. Bacteria from the mother's intestine migrate into the amniotic fluid, where they are swallowed by the embryo. After birth, intestinal bacteria enter the mother's milk via the entero-mammary pathway through macrophages from the intestine via the blood and then colonize the newborn's intestine. If the mother has an unhealthy microbiome, there is a risk of neurodermatitis and allergies later in life.

Aging

The diversity of the microbiome is crucial for healthy aging. Due to an increasingly one-sided diet in old age, there is less and less diversity. This process is favored by the excessive administration of pharmaceuticals and antibiotics. Especially in people with Alzheimer's disease, diversity is extremely limited and shows a typical pattern.

Antibiotics

The indiscriminate use of antibiotics as medicines and in factory farming has generally led to reduced diversity in young and old, resulting in an increase in chronic diseases. If antibiotic therapy cannot be avoided, it must be accompanied by a probiotic therapy.

Viruses

Viruses are an important topic. At the moment SarsCov2 dominates the discussion, but we should not forget the many other pathogenic viruses like Epstein-Barr viruses, dengue, influenza etc. Certain strains of bacteria are able to prevent viruses from entering the body, inhibit docking at receptors, and even produce virucidal substances. We use this capability in therapy.

Examination

The microbiome is assessed via PCR, which makes it possible to detect hundreds of bacterial strains, including fungi and parasites.

Therapy

We treat using specific strains of bacteria depending on the indication (probiotics), prebiotics, nutritional supplements, and nutritional counseling.



The Role of Thai-based Herbal Medicine in COVID-19

[The effect of *Andrographis paniculata* capsule in reducing the severity of complications associated with coronavirus infection in COVID-19 patients in Mae Fah Luang University Medical Center Hospital: A retrospective study]



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ABSTRACT

Andrographis paniculata (Burm.f.) Wall.ex Nees is known as “King of Bitter”, which belongs to the Acanthaceae family. It is an important medicinal plant for treating various ailments such as the common cold, diarrhea, and fever due to several infectious causes. A major bioactive component of *A. paniculata* is andrographolides, a diterpene lactone. According to the Guidelines for clinical practice, diagnosis, treatment and prevention of healthcare-associated infection in response to patients with COVID-19 infection 2021 of Thailand had recommend using *A. paniculata* capsule in COVID-19 patient with mild symptoms, no risk factor for severe COVID-19 infection, no contraindication of using *A. paniculata* capsule, with calculated dosage of andrographolide is 180 mg/person/day in 3-4 times/day before meal for 5 consecutive days. In this retrospective study, we enrolled 110 patients (mean age: 31.44 ± 9.24 years; 51.82% males and 48.18% females) with laboratory-confirmed COVID-19 admitted to Mae Fah Luang University Medical Center Hospital, Chiang Rai, Thailand from June to September 2021. Demographical, clinical, and symptoms on admission were collected; complications and outcomes were followed up for 5 days. The study outcomes were assessed using descriptive statistics and repeated-measures ANOVA. Among mild COVID-19 of 110 patients with lab-confirmed COVID-19, 70% presented with respiratory symptoms (cough, runny nose, sore throat, dyspnea), 16.36% had gastrointestinal symptoms (diarrhea), 5.45% had dermatological symptoms (rash), 22.73% had neurological (headache), and 57.27% had other non-specific symptoms (fever, conjunctivitis, myalgia, disturbances of smell or taste). Among those patients who were hospitalized at



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Mae Fah Luang University Medical Center Hospital, Chiang Rai, Thailand and taking *A. paniculata* capsule for 5 consecutive days (N = 110), the symptoms score of respiratory symptoms (cough, runny nose, sore throat and dyspnea), gastrointestinal symptoms (diarrhea), neurological (headache), and other non-specific symptoms (fever, myalgia and disturbances of smell or taste) were significantly decreased from baseline with $p < 0.05$. Therefore, this study suggests the potential of *A. paniculata* capsule to relieve various symptoms in patients with COVID-19 infection.

Abstract

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Abstract
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Abstract

Open Access

Effectiveness of Huangqi Guizhi Wuwu Decoction on Adult Diabetic Neuropathic: A Systematic Review

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ABSTRACT

Introduction: Chinese herbal medicine formula Huangqi Guizhi Wuwu decoction (HGWD) is commonly used for arthritis, spondylosis, stroke, etc. Previous research showed that HGWD also suggested diabetic neuropathic (DNP) treatment

Objective: To evaluate the effectiveness and safety of HGWD in treating adult DNP.

Methods: Six databases, including Pubmed, Cochrane Library, EMBASE, China Network Knowledge Infrastructure (CNKI), Wanfang, and Chinese Scientific Journals were searched from their inception to May 2022. Only randomized control trials (RCTs) that evaluated HGWD for adult DNP were included in this review. Two investigators independently evaluated and extracted the data.

Results: A total of 181 RCTs and 15,657 participants were included in the review. In these studies, oral HGWD single used or HGWD combined with oral drugs were compared with oral methylcobalamin, epalrestat, etc., single used. The outcomes of effective rate and adverse events were analyzed. The effective rate of HGWD is higher than other pharmacotherapies (RR:4.50, 95% CI [4.09,4.96], P < 0.05). The results showed the intervention group had fewer adverse events (RR: 0.54, 95% CI [0.34, 0.86], P < 0.05), which included nausea, abnormal liver and kidney function, gastrointestinal overreaction, etc.

Conclusion: The meta-analysis results showed that HGWD is effective and brings fewer adverse events to adult DNP. It is recommended in treating DNP, but further research about the mechanism is necessary for deeper understanding.

Keywords: *Huangqi Guizhi Wuwu decoction; Chinese herbal medicine formula; diabetic neuropathic; systematic review, Meta-analysis*



Therapeutic Efficacy of Knee Acupuncture added Sanyinjiao (SP6) Acupoint for Pain Reduction in Knee Osteoarthritis Patients

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ABSTRACT

Introduction: Osteoarthritis is one of the musculoskeletal changes in the elderly, which is involved in the degradation of the cartilage, and bone remodeling in the affected joint. Inflammation was also associated with osteoarthritis of the knee. Therapeutic strategies that alleviate inflammation could reduce pain and improve knee motion. Previous studies have reported that traditional knee acupuncture could reduce pain and improve knee motion. However, the therapeutic efficacy of traditional knee acupuncture added Sanyinjiao (SP6) acupoint is still unknown.

Objective: This study was to investigate the effects of knee acupuncture added Sanyinjiao (SP6) acupoint on pain, inflammation, and knee joint motions. We hypothesized that acupuncture could reduce pain, inflammation and lead to improved joint motions.

Methods: 15 patients were enrolled. All patients received acupuncture for 10-sessions, 2 times a week for 5 weeks. Pain visual analog scale (VAS), knee motion, and plasma hs-CRP were determined as a baseline and at the end of the treatment protocol.

Results: 78% were female, aged ranging from 60 to 75 years old. After 10 treatment sessions, an improvement in the flexion and extension were found accordingly with a reduction in knee pain. In addition, plasma hs-CRP was significantly decreased (1.64 ± 0.89 mg/dL vs. 4.32 ± 0.56 mg/dL at baseline, respectively).

Conclusion: Knee acupuncture added SP6 point could improve knee motion and decrease pain through a reduction of inflammations.

Keywords: *knee osteoarthritis; acupuncture; inflammation; pain; knee motion*



Anti-inflammatory Effect of Folk Remedy for Hemorrhoids Treatment

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ABSTRACT

Introduction: Mr. Kitti Nakun, a well-known folk healer in Ubon Ratchathani province. Nowadays, hemorrhoids are one of the many diseases that he treats patients with herbal medicine. The hemorrhoids treatment remedy of Mr. Kitti Nakun consisted of 4 herbs including *Salacia chinensis* L., *Salacia verrucosa* Wight., *Curcuma comosa* Roxb., and *Cissus quadrangularis* L.

Objective: The objective of this research was to study the anti-inflammatory activity of extract from a hemorrhoid treatment remedy of Mr. Kitti Nakun.

Methods: The remedy was extracted with water and freeze-dried. Then, the extract was tested for cytotoxicity on macrophages (RAW264.7 cells). The anti-inflammatory effect was examined via the nitric oxide production by using Griess reagent and the inflammatory-related genes by using semi-quantitative reverse transcription and polymerase chain reaction technique in lipopolysaccharide-induced cells.

Results: The results showed that the extract had a low cytotoxicity with the cell growth inhibitory concentration (IC₅₀) greater than 1000 µg/mL. Moreover, the anti-inflammatory activity test with Griess reagent demonstrated that the extract at a concentration of 200 µg/mL was able to inhibit nitric oxide secretion by 46.38%. In addition, the extract at concentration of 50-200 µg/mL were able to inhibit cyclooxygenase-2 and tumor necrosis factor- α gene expression in a dose dependent manner.

Conclusion: The information of this study is very important and useful in promoting the wisdom of folk healers to be recognized and further developed.

Keywords: folk remedy, anti-inflammation, hemorrhoids, herbal medicine



The Protective Effect of Thai Herbal Wattana Formula on Chronic Cerebral Hypoperfusion Induced by Bilateral Common Carotid Artery Ligation

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ABSTRACT

Introduction: As the association between cognitive impairment and cerebral hypoperfusion in different types of dementia has been established, it is important to unravel the hypoperfusion-related neurodegenerative and cognitive correlates. Components of Thai herbal Wattana formula (WNF) have been proven to exhibit anti-oxidant and anti-inflammatory actions and to reduce amyloid pathology-related neurotoxicity and cognitive dysfunction.

Objective: We aimed to explore neuroprotective effects of WNF in the 2-VO (2 vessel occlusion) model and to examine its therapeutic potential as an alternative option for chronic cerebral hypoperfusion (CCH).

Methods: The adult male Wistar rats (n=5/group) underwent bilateral common carotid artery ligation. The WNF recipe (300 mg/kg) was administered orally after surgery for 60 days. The Morris Water Maze test was performed between day 61 and 70 followed by Cresyl Violet staining of the brain tissue on day 71.

Results: We found that WNF tended to aid memory retrieval ($p < 0.6396$, 2-VO versus 2-VO + WNF) but did not improve memory acquisition after CCH ($p < 0.0997$, 2-VO versus 2-VO + WNF). The impaired survival of neurons was improved by WNF in the right CA3 subregion ($p < 0.05$ 2-VO versus 2-VO + WNF).

Conclusion: This will be the first time that our study explored the potential therapeutic efficacy of WNF and opened a therapeutic window to ameliorate neurodegeneration in conditions that cause CCH such as vascular dementia.

Keywords: chronic cerebral hypoperfusion; Thai herbal Wattana formula; WNF; Alzheimer's disease; neurodegeneration.



The Effects of Orange, Sweet Basil, and Dok Mok Essential Oils Inhalation on Emotional State and Autonomic Nervous System

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ABSTRACT

Introduction: Chronic stress can be detrimental to both physical and mental health. Since essential oils (EOs) from herbs have been shown to activate the nervous system by sending an electric signal to limbic system modulating behavioral and emotional responses, and reversing a stress-induced change in both nervous system and hormone secretion. EOs from three Thai native medical herbs - *Citrus reticulata* Blanco (Orange), *Wrightia religiosa* (Dok Mok), and *Ocimum basilicum* L. (Sweet Basil) - are also used as aromatherapy to alleviate stress in Thai society for ages. Orange EO, Dok Mok EO, and Sweet Basil EO contain limonene, linalool, and linalool & eugenol respectively which are reported anti-stress, anti-anxiety, and anti-depression effects. **Objective:** This study aimed to investigate the effects of the three herbs EOs on the autonomic nervous system (ANS) and emotional states.

Methods: In this study, Orange EO is extracted by a cold press machine. Dok Mok EO is extracted by solvent extraction. Sweet Basil EO is extracted by steam distillation. Twelve healthy volunteers consented to participate in the experiment, and were assessed blood pressure and heart rate to determine the arousal level of the ANS. Besides, the participants filled out a questionnaire on emotional states before and after the 20-minute inhalation of aroma samples. Data were analyzed using paired sample t-tests. Means of the blood pressure, heart rate, and emotional states of subjects were compared before and after the inhalation.

Results: The results showed that, in terms of ANS, the heart rate mean was significantly decreased after the inhalation ($p < 0.05$) while the blood pressure mean did not show any statistically significant decrease. The mean of emotional state scores inputted by the subjects was significantly improved ($P < 0.05$).

Conclusion: This study demonstrated that aromatherapy from Orange, Dok Mok, and Sweet Basil seems to have effects on ANS regulation and positive emotional states. However, further studies are needed.

Keywords: essential oil; stress; orange; sweet basil; dok mok



Role of Intravenous Glutathione and High-dose Vitamin C in Adult Acute Lymphoblastic Leukemia Adjunctive Therapy

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) is rare and has poor prognosis in older adults. The challenging aspect of ALL management in adults is that the patient and leukemic factors are imperative components when designing the treatment regimen. Intravenous (IV) therapy increases bioavailability beyond oral administration and is one of the essential treatments utilized among licensed Naturopathic doctors (NDs). Glutathione (GSH) plays an important role in multiple pathways of programmed cell death, particularly in apoptosis. High-dose intravenous vitamin C (HDIVC) enhances immune function. This case report discussed an adult patient with ALL who received IV therapy while waiting for allogeneic stem cell transplantation (allo-SCT), one of the standard treatments to achieve remission.

Objective: The aim was to evaluate the effectiveness of IV GSH and HDIVC for an adult with ALL as adjunctive therapy.

Methods: A 43-year-old Hispanic female with an ALL relapse. The patient was a good candidate for allo-SCT. She was simultaneously treated with oral chemotherapy, while receiving adjunctive therapy, including multi-nutrients IV drips followed by a GSH push, HDIVC, nutraceuticals, botanicals, homeopathy, and nutritional counseling. We evaluated the effectiveness of the treatment regimen using subjective and objective measures: (1) blood pressure, (2) blood glucose, (3) energy rating scale, and (4) quality of life.

Results: IV multi-nutrient drip with GSH push alternated with HDIVC administered bi-weekly helped reduce fatigue and improved quality of life in this patient. No adverse events reported. However, the complete remission of ALL was delayed mainly due to the lack of insurance and the uniquely challenging pandemic related to coronavirus disease 2019 (COVID-19).

Conclusion: IV GSH and HDIVC are proven to be effective and safe adjunctive treatments to improve quality of life and extend life of an adult patient with ALL. This treatment regimen deserves consideration as an adjunctive therapy for similar patients.

Keywords: acute lymphoblastic leukemia; IV therapy; high dose vitamin C; glutathione



The Effect of External-Focused Attention Using the Prototype Model of SCF Innovation (Speedy Colors Fitting) on Postural Control in Healthy Young Adults

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ABSTRACT

Introduction: Postural control is the ability to control the body's position and is an important fundamental factor for daily living activities, which requires coordination between an object and upper extremity while maintaining the body's position depends on postural control. The prototype model of SCF (Speedy Colors Fitting) innovation was invented to train postural control by stimulating the coordination between eyes and hands for enhancing cognition through using symbols and colors as external stimuli simultaneously with using the random pattern of colors as choice reaction time.

Objective: This study aimed to study the effect of external-focused attention using a prototype model of SCF (Speedy Colors Fitting) innovation on postural control.

Methods: This study is a quasi-experimental design. Ten healthy participants were recruited, and all were trained in postural control by the SCF innovation prototype model. Participants completed static balance in multiple conditions, including a quiet stance with eye closed and eye open. Dynamic balance tests were performed on the functional reach and the timed-up and go test with a wearable movement sensor (APDM). Data were recorded as outcomes; center of pressure (COP) path length, COP velocity, unilateral duration, turn angle, turn duration, and sit-to-stand duration before and after training.

Results: After training with the prototype model of SCF innovation, the COP path length of static balance in each condition did not significantly increase. The dynamic balance decreased with no statistical significance. COP velocity increased in static balance, dynamic balance, and functional reach with no statistically significant difference. Timed-up and go test variables decreased after training with no statistical significance (p -value = 0.092). The functional reach test found that participants could reach further after training (p -value = 0.139). However, there was no statistically significant difference between before and after training.

Conclusion: Providing external-focused attention by prototype model of SCF innovation on postural control in healthy young adults could enhance the ability to control the body's position while in motion or dynamic balance.

Keywords: *external-focused attention; postural control; center of pressure*

**Abstract
for oral presentation**

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Clinical Research on the Treatment of Hyperthyroidism with Chaihu Guizhi Longu Muli Decoction

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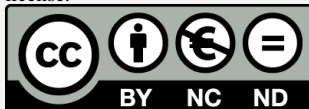
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ABSTRACT

Introduction: The basic formula of Chaihu Guizhi Longgu Muli Decoction (CGLMD) is derived from Xiao Chaihu Decoction combined with Guizhi and Longgu Muli Decoction. At present, many studies in China have proved that the use of CGLMD combined with Western medicine in the treatment of hyperthyroidism is more effective and can reduce the side effects of Western medicine.

Objective: To observe the clinical effects of CGLMD combined with antithyroid drugs (Methimazole) in the treatment of hyperthyroidism and safety of CGLMD.

Methods: 62 patients were included in the OPD of Endocrinology, First Affiliated Hospital of Guangzhou University of Chinese Medicine from April 2019 to December 2019. Patients are randomly divided into control and treatment groups. Patients in the control group were treated with Methimazole, and the patients in the treatment group were treated with CGLMD combined with Methimazole. The duration of treatment was 4 weeks and 8 weeks for continuous observation. Thyroid function (TSH, FT3, FT4), liver function (AST, ALT) and blood analysis (WBC, NEU), self-designed clinical symptom observation scale, and clinical symptom registration form were observed before and after treatment.

Results: 1. Total score of TCM in treatment group decreased more than control group ($P < 0.05$). In 29 symptoms that were observed, the treatment group improved 21 symptoms, including mental depression, palpitation, thirst, etc. ($P < 0.05$). The control group effectively improved depression and insomnia ($P < 0.05$). The total effective rate of the two groups was 78.2% and 26.7% respectively, and the difference was statistically significant ($P < 0.05$).

2. Thyroid function of patients in both groups was reduced ($P < 0.05$), and the TSH between 2 groups not statistically different ($P > 0.05$), while FT3 and FT4 were statistically difference ($P < 0.05$).

3. 3 cases of abnormal liver function were found in the control group, with no other adverse reactions. There was no obvious abnormality in blood analysis between two groups.

Conclusion: The results proved that the combination of CGLMD with antithyroid drugs can obviously alleviate the clinical symptoms of patients although the effective improvement of TSH level was not obvious, and the treatment was safe for patients.

Keywords: Chaihu Guizhi Longgu Muli Decoction; hyperthyroidism; clinical efficacy



Abstract

Open Access

Impact of Scoliosis on Gait Characteristics: A Case Study of A Child with Spastic Diplegic Cerebral Palsy

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ABSTRACT

Introduction: Neuromuscular scoliosis is a common musculoskeletal problem for people with cerebral palsy. Neuromuscular scoliosis develops due to poor muscle control and spasticity. Scoliosis is not only a spinal deformity, but also possibly leads to the development of gait pathology and impact on postural control and the quality of life.

Objective: To verify the impact of scoliosis on gait characteristics of a child with spastic cerebral palsy.

Methods: To comply with the proposed objectives, three children with spastic diplegic cerebral palsy, GMFCS level III, participated in this study. One case was a 12-years old with scoliosis, and another two cases were boys without scoliosis, aged 7 and 8 years. The participants performed a 10-Meter Walk Test. The main outcome variables including gait cycles, gait speed, cadence, and stride length were measured by OPAL accelerometer, which were analyzed using Mann-Whitney U Test.

Results: The results showed no difference in gait speed, cadences, and stride length among the child with spastic diplegic cerebral palsy with and without scoliosis. However, for the gait cycles, the stance phase and double support of the child with scoliosis trended to be longer than those without scoliosis.

Conclusion: Further investigations are certainly need to assess the impact of scoliosis on gait characteristics in children with spastic cerebral palsy.

Keywords: *gait; Scoliosis; diplegia; cerebral palsy*



Immediate Effect of Thai Massage on Calf Muscle Flexibility and Postural Sway in Overweight Children: Pilot Study

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ABSTRACT

Introduction: Nowadays, overweight children are increasing and possibly impact on postural control. The excessive body weight reacted to postural perturbations and various body systems. Maintaining posture is essential for daily activities. However, the intervention to improve postural balance in overweight children is limited.

Objective: To explore the immediate effects of Thai massage on postural balance by recording the trajectory of the center of pressure (COP) in overweight children.

Methods: Twenty Thai children who are 10 – 12 years old with overweight (BMI from 85th to 94th percentile) were recruited from the school in Chiang Rai province and then categorized into treatment and control groups. Postural sway was examined using a force plate (Zebris Medical, Isny, Germany). Gastrosoleus muscle flexibility was measured by weight bearing ankle lunge test. The treatment group received a Thai massage for 30 minutes on the area of both feet, legs and hips and the control group rested on bed.

Results: There were no statistically significant differences between groups of postural sway. The flexibility of gastrosoleus muscle was significantly differences between groups ($p < 0.05$).

Conclusion: Thirty minute of Thai massage sessions showed immediate improvement of muscle flexibility that is important for postural control. However, there are some limitations of this study including small sample size and convenience sampling.

Keywords: *Thai massage; postural sway; postural balance; muscle flexibility; overweight children*



Relationship between Balance Ability and Ankle Function in Patients with Diabetes Mellitus and Peripheral Neuropathy

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ABSTRACT

Introduction: Peripheral neuropathy is the main complication resulting in ankle muscle weakness in patients with diabetes mellitus. The dysfunction leads to impair balance control and increase risk of falls in these individuals. Recently, there is limited evidence regarding the correlation between balance ability and ankle function in patients with diabetes mellitus and peripheral neuropathy (DMPN).

Objective: A cross-sectional study to investigate the correlation between balance ability and ankle muscle function in patients with DMPN.

Methods: 34-DMPN participants were evaluated for balance ability using one-leg stance test (OLST) with eye-open and eye-close conditions. Then, they were assessed ankle muscle strength using a hand-held dynamometer (HHD) in 4 muscle groups. The relationship was investigated using Spearman's Rank Correlation Coefficient.

Results: The average age of DMPN participants was 62.64 ± 5.21 years old, and the score of peripheral neuropathy using 5.07 monofilament was 4.11 ± 2.68 points. The findings found a significant correlation between both OLST and the ankle plantar flexor muscle group (0.439, $p=0.009$ for OLST eye-open, 0.491, $p=0.003$ for OLST eye-close). In addition, a significant correlation was found in OLST eye-close condition for the ankle invertor muscle group (0.439, $p=0.009$) and evertor muscle group (0.449, $p=0.008$).

Conclusion: The findings showed the importance of ankle muscle function to balance control in DMPT. However, further studies should compare the result with a healthy in order to confirm the relationship between peripheral neuropathy and muscle activation in DMPN.

Keywords: *Peripheral neuropathy; ankle strength; ankle function; balance control*



Abstract

Open Access

Surface Anatomical Positioning of the 2nd and 3rd Shoulder Signal Points of Court-type Thai Massage in Male Humans

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ABSTRACT

Introduction: The 2nd and 3rd shoulder signal points are on the neck region of the human body which are essential for the examining and massaging of symptoms in Thai traditional medicine.

Objective: This research aimed to identify the 2nd and 3rd shoulder signal points on the x and y - coordination of male human body.

Methods: The thirty healthy male volunteers (18-22 years, 65-75 kg. weight, 170-179 cm. height and BMI = 18.5-24.9) were included in this research. They were divided into groups A B C and matched with the reference persons A B C consecutively. After the 2nd and 3rd shoulder signal points were identified by the reference persons, the lateral aspect of the mastoid process was tagged as the surface anatomical landmark. Then the photograph was recorded. The 2nd and 3rd shoulder signal points were measured with the ImageJ software.

Results: The lateral aspect of mastoid process was the origin of the x and y coordination. The average distance of 2nd shoulder signal point was at $x = 0.7 \pm 0.6$ cm.; $y = -8.2 \pm 0.9$ cm. in group A, $x = 0.0 \pm 1.2$ cm.; $y = -8.7 \pm 0.9$ cm. in group B, and $x = 2.6 \pm 1.4$ cm.; $y = -14.5 \pm 1.1$ cm. in group C. The distance of 3rd shoulder signal point was $x = 0.8 \pm 0.3$ cm.; $y = -9.2 \pm 1.0$ cm. in group A, $x = 0.8 \pm 0.9$ cm.; $y = -11.5 \pm 1.3$ cm. in group B, and $x = 2.3 \pm 1.4$ cm.; $y = -15.2 \pm 1.1$ cm. in group C. The average of intersection of 2nd and 3rd shoulder signal points were at $x = 1.1 \pm 1.6$ cm.; $y = -10.5 \pm 3.0$ cm. and $x = 1.3 \pm 1.2$ cm.; $y = -11.9 \pm 2.7$ cm. consecutively.

Conclusion: The signal points of court-type Thai massage could be manifested into the universal form and the surface anatomical landmark should be considered for identifying accurately.

Keywords: Thai traditional medicine; court-type Thai massage; signal shoulder points; surface anatomical landmark; coordinate line



Application of Tonifying Kidney, Invigorating Qi, and Promoting Blood Circulation in Common Gynecological Diseases

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ABSTRACT

The concepts and theories of tonifying kidney, invigorating Qi, and activating blood circulation originate from “Nei Jing”, which have become the essence of traditional Chinese medicine theory, providing theoretical support for the establishment of new methods for the treatment of various obstetrics and gynecology diseases, as the disease spectrum has broadened and the understanding of diseases has deepened. These three treatments can be used separately or in combination.

Our research team has flexibly implemented the technique of invigorating the kidney, nourishing qi, and activating blood circulation to clinical therapy. For example, in the case of embryo implantation failure, Bushen Antai recipe, which means tonifying kidney and miscarriage prevention, could be used to promote the endometrial receptivity, angiogenesis, and immunological tolerance at the maternal-fetal interface, thereby resulting in a better pregnancy outcome. As for the premature ovarian failure, Siwu Tang, which can nourish the blood and activate the circulation, has been taken as the basic recipe to promote ovarian angiogenesis and the function of granulosa cells, thus improving the follicular development and restoring the ovarian reserve. Regarding the polycystic ovary syndrome, Jiaotai Siwu decoction, which clears away the heart fire, warms the kidney, and relieve the water retention, is used to coordinate the heart and kidney and alleviate the abnormal metabolism, endocrine, and chronic inflammation state, so as to repair the ovarian ovulation.

Over the last 10 years, our study group has been dedicated to the application and mechanism exploration of principles of tonifying kidney, invigorating Qi, and activating blood circulation in regulating menstruation, embryo implantation, and protection of the embryo development, which enrich the scientific connotation of traditional Chinese medicine in treating gynecological diseases.

Keywords: *application; tonifying Kidney; invigorating Qi; gynecological Disease*



Abstract

Open Access

Study of Acupuncture Improving Oocytes Quality of Poor Ovarian Responders in IVF

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ABSTRACT

Introduction: Poor Ovarian Response (POR) occurs among women undergoing In Vitro Fertilization-Embryo Transplantation (IVF-ET), it affects the quantity and quality of retrieved follicles and embryos. Few approaches were proved beneficial for POR patients, while acupuncture might be an effective alternative and complementary therapy.

Objective: This random controlled trial (RCT) was conducted to investigate the effect of acupuncture on oocytes quality of Poor Ovarian Response (POR) patients in IVF.

Methods: POR patients were randomly assigned into acupuncture + IVF group and IVF group. The study period was 20 weeks, including baseline for 1 week, acupuncture treatment for 8 weeks, IVF cycle for 3 weeks and an 8-week follow-up. Acupuncture was performed in the acupuncture plus IVF group, from two menstrual cycles before IVF cycle, three times a week, except the menstrual period, until the HCG injection day, besides, IVF group received no acupuncture. Both groups received a micro stimulation program for follicle stimulation in IVF. The main outcomes were mature oocytes rate and high-quality embryo rate, secondary outcomes included number of eggs retrieved, number and rate of fertilization, rate of high-quality embryos, number and rate of available embryos, Gn dosage and GN days. Above outcomes were evaluated on the third day after follicles were retrieved.

Results: The main outcomes: the number of mature oocytes in the two groups was similar ($P > 0.05$); the high-quality embryo rate of acupuncture plus IVF group was significantly higher than that of IVF group ($P < 0.01$).

Secondary outcomes: Number of high-quality embryos & rate of available embryos in acupuncture + IVF group were significantly higher than those in IVF group ($P < 0.05$); the number of retrieved eggs, fertilized embryos, available embryos, the rate of fertilized embryos, the dosage and days of Gn were similar between two groups ($P > 0.05$).

Conclusion: Acupuncture improved the oocytes quality of POR patients in IVF, mainly manifested as increased number and rate of high-quality embryos.

Keywords: *acupuncture; poor ovarian response; IVF; oocyte Quality*



The Effect of Muscle Fatigue on Activation Patterns of the Biceps Femoris and Semitendinosus Muscles in Healthy Soccer Players Aged 19 to 35 Years

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ABSTRACT

Introduction: Hamstring injuries are among the most common injuries in soccer players. Especially the danger of hamstring injuries increases in the final third of each half, with the biceps femoris muscle being highly susceptible, but currently there is no supporting evidence in soccer players. It is important to investigate to provide a basis for further clinical investigations in order to reduce the risk of hamstring injuries.

Objective: To investigate a soccer-specific muscle fatigue protocol has an effect on the activation patterns of the biceps and semitendinosus muscles and correlates with the subjectively perceived exertion of the soccer players.

Methods: 19 healthy soccer players aged 19 - 35 years were included and underwent a standardized procedure that included: (1) performance of the nordic hamstring curls (NHC) and a 10m sprint (pre-test), (2) a soccer-specific muscle fatigue loading protocol for 30 minutes with subsequent rating of perceived exertion (RPE), (3) re-assessed like pre-assessment (post- test). Electromyography (EMG) data were recorded the biceps and semitendinosus muscles of both legs. Two parameters were analyzed: the peak amplitude during the NHC and the mean amplitude during the 10m sprint.

Results: The RPE score (9.5, $p < 0.001$) and 10m sprint shows significant differences between pre- and post- test for the biceps (standing leg: -82.45, kicking leg: -81.77; $p < 0.01$) and semitendinosus muscle (standing leg: -60.08, $p = 0.001$); kicking leg: -65.30, $p = 0.03$). Significant correlation exists between RPE score and biceps muscles at post-test (standing leg: $r = -0.54$, kicking leg: $r = -0.51$; $p < 0.05$).

Conclusion: Muscle fatigue leads to significant changes in the activation behavior of the hamstring muscles. In particular, the biceps muscle shows a correlation with perceived exertion, the higher the exertion, the lower the muscle activity. This may help explain the higher prevalence of the biceps muscle in hamstring injuries and offers a basis for further clinical investigations.

Keywords: muscle activity; hamstrings; electromyography; muscle fatigue; soccer

Relationship between Predisposing Factors and Risk of Work-Related Musculoskeletal Strain among Poultry Slaughterhouse Workers – A Cross Sectional Study

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ABSTRACT

Introduction: In Thailand, the poultry slaughterhouse industry is one of the largest sectors of the food industry which continues to grow as well as the number of poultry workers that increase significantly. The poultry workers are exposed to repetitive movements and prolonged standing for entire working hours, which may predispose to the risk of work-related musculoskeletal strain (WMS) especially at the upper limb and neck region.

Objective: To evaluate the relationship between predisposing factors and the risk of WMS among poultry slaughterhouse workers.

Methods: A cross-sectional observational study was conducted in Thailand among 78 poultry slaughterhouse workers. A self-reported profile of the workers were collected. Rapid Upper Limb Assessment (RULA) was conducted to examine the risk of WMS to upper limb and neck by a physiotherapist. The total RULA score at 7 (high risk level) was used as a cut off point for investigating the relationship between the predisposing factors and WMS risk by using the binary logistic regression analysis (AMSEC-64EX-112).

Results: A total of 78 participants, 48.7% (N=38) men, and 51.3% (N=40) women participated in the study with a mean age of 39.12±9.33 years. The mean work experience and working hours/day were 4.33±4.04 years, and 10.42±1.59 hours, respectively. 37.2% (N=29) were smokers, and 77% (N=60) worked more than 8 hours/day. About 11.5% (N=9) worked in slaughtering task, 41% (N=32) worked in evisceration task, and 47.4% (N=37) worked in cut-up task. 80.7% (N=63) had RULA score at 7. Predisposing factors: working hours (more than 8 hours) ($R^2=0.137$, $\beta=13.33$, $p=0.001$), evisceration ($R^2=0.145$, $\beta=19.33$, $p=0.001$) and cut-up task ($R^2=0.145$, $\beta=10.33$, $p=0.005$), and smoking ($R^2=0.05$, $\beta=0.31$, $p=0.048$) were significantly associated with RULA.

Conclusion: This study identified certain predisposing factors such as working hours, evisceration and cut-up task, and smoking related to risk of WMS to upper limb and neck region among poultry slaughterhouse workers.

Keywords: poultry slaughterhouse workers; predisposing factors; work-related musculoskeletal strain



Measuring Movements 24 Hours per Day in Older Persons Living in a Nursing Home - A Pilot Study

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ABSTRACT

Background: Physical activity plays a crucial role in maintaining function and movement in daily life among nursing home residents. Modern technology allows research on physical activity and gait parameters measured with mobile devices and for a long time. The purpose of this study was to describe physical activity, sedentary time and gait parameters among nursing home residents.

Methods: Five participants, ranging in age from 75 to 98 years old were recruited at a nursing home in Lund, Sweden to control the environment (median 88 years old). Over a two-week period, the Inertial Measurement Unit (IMU) device was used to measure various position changes and gait function for 24 hours. We specifically measured lying down, sitting and standing time, as well as gait parameters such as walking speed, step length, step regularity, and number of steps per day. The variables were described using mean, median, minimum-maximum, and percentage values.

Results: The median of mean values for all lying down at 6 hours and 17 minutes. In particular, side-lying position was 22 hours and 22 minutes, including sitting position of 7 hours and 39 minutes among nursing home residents. The gait parameters were represented by a median of mean value elements of walking speed was 0.35 m/s, step length was 18.55 cm, and the number of steps was 65 steps per day. Nevertheless, the median of mean value for step regularity was illustrated in high percentages at 71.43.

Conclusion: Time spent in lying down and sitting positions for more than three hours indicated lack of physical activity and high level of sedentary behavior in nursing home residents and also had a poor walking ability corroborating the logical theory of aging. Hence, the findings are able to enhance physical activity and gait function among nursing home residents further.

Keywords: *physical activity; IMU device; walking speed*



Occurrence of Musculoskeletal Health Complaints and Corresponding Risk Factors among Music Students and Non-music Students – Results from a Prospective Cohort Study

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ABSTRACT

Introduction: Musculoskeletal complaints (MHC) in professional musicians are common but a lack of prospective studies in the field of music medicine, identification of causal relations in the etiology of musculoskeletal disorders in instrumental musicians is challenging.

Objectives: A prospective cohort study was launched with 1) determining the prevalence of MHC among music students 2) to compare the physical and mental health of music students and non-music students at the start of the study 3) identify risk variables for MHC, and 4) develop an MHC prediction model in musicians.

Methods: The measurements consisted of a self-designed questionnaire addressing history of pain, localization of pain, habits, practicing and sports, validated questionnaires measuring health related QuOL (SF12), stress symptoms (SCI), and performance anxiety (KMPAI) and physical examination including core stability, hypermobility, cervical range of motion, mechanosensitive. Occurrences (incidence and prevalence) of MHC were recorded monthly by online questionnaires.

Results: 278 are included for the cross-sectional comparison and 149 for the longitudinal analysis so far. The monthly and yearly prevalence of MHC was 27% and 56% respectively. Yearly incidence rate was 29%. The number of stress symptoms and mechanosensitive were considerably increased ($p < 0.05$) in music students compared to non-music students, but physical and mental health, physical activity, and general cervical CROM were dramatically decreased ($p < 0.05$). Longitudinal analysis, significant risk factors for the development of MHC within one year were being a music student, increased bodily pain, history of complaints, higher number of stress symptoms, reduced physical and mental health, and history of smoking ($p < 0.05$). Furthermore, a set of mainly pain and physical health related variables including smoking showed good ability in predicting MHC (AUC=0.74).

Conclusion: In further cohorts the results need to be replicated. Additionally, occurrences and risk factors of MHC will be analyzed body region and instrument specific

Keywords: music health; cohort study; risk factors; prediction



Abstract

Open Access

Effects of Modified-OTAGO on Body Composition in Older Adults during COVID-19 Outbreak: A Preliminary Study

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ABSTRACT

Introduction: Aging is characterized by an unavoidable decline in body composition, including changes in muscle mass, muscle quality, and adiposity. These compartments contribute to falls, a major health issue that causes serious injuries and death. The COVID-19 outbreak causes long-term quarantine, which limits outdoor activity leading to physical inactivity. As a result, it worsens body composition declination, raising the risk of falls. The Otago Exercise Program (OEP) is widely used to prevent falls. However, it is time-consuming and requires additional walks to get better efficacy.

Objective: This study aims to investigate the effects of modified-OEP on body composition in community-dwelling older adults.

Methods: This study was a double-blinded randomized control trial. Participants aged 60-80-year-olds were randomly assigned to the modified-OEP or control group. The modified OEP group performed a 60-minute session consisting of 40-minutes OEP and 20-minutes walking, while no intervention was assigned to the control group. Body composition was assessed before and 6 weeks after intervention using Dual Energy X-ray Absorptiometry (DEXA). Repeated measures two-way ANOVA was employed for statistical analysis.

Results: After 6 weeks of interventions, the modified-OEP group had a significant decrease in leg fat mass ($p = 0.0008$), total fat mass ($p = 0.0036$), and total tissue percent fat ($p = 0.0320$). These observations were not observed in the control group, indicating the efficacy of the modified-OEP. In comparison between groups, the modified-OEP did not show a statistical difference from the control. However, we observed an improvement trend in lean mass and fat-free.

Conclusion: We demonstrated that the modified-OEP can significantly reduce fat in as little as six weeks. Further investigation should explore the long-term efficacy of such a program.

Keywords: OTAGO exercise; older adults; body composition



Prevalence and Associated Factors of Forward Head Posture among Physical Therapy Students

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ABSTRACT

Introduction: The students in the physical therapy program, however, have the activity during study hour, but they have to spend many hours studying in front of the computer which leads to the forward head posture.

Objective: The aim of this study is to investigate the prevalence and related factors of the forward head posture.

Methods: This cross-sectional study was conducted in 98 physical therapy students. The participants were asked about personal data, the last day and last week screen time duration which were collected by phone, and the physical activity by Thai Global Physical Activity Questionnaire version 2 (GPAQ). After that they measured the Tragus wall distance (TWD) to diagnose the forward head posture.

Results: The results show that the prevalence of forward head posture is 65.31 and the factors associated with forward head posture were screen time (p-value < 0.01) and physical activity (p-value < 0.05).

Conclusion: There was a high prevalence of forward head posture in physical therapy students in Mae Fah Luang University which were associated with high screen time and low physical activity.

Keywords: *forward head posture; prevalence: associated factors*

Knowledge, Awareness, and Practice Regarding Pesticide Exposure and Health Symptoms among Farmers

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ABSTRACT

Introduction: Pesticides are widely used to control agricultural insects in Thailand. Farmers have an opportunity to be exposed to high doses of pesticides. The improper pesticide application may also have resulted in adverse health effects.

Objective: This study aimed to investigate the association between farmers' knowledge, awareness, and practices regarding pesticide use and health symptoms related to pesticide exposure.

Methods: A cross-sectional study was conducted among farmers in Chiang Dao district, Chiang Mai province. A validated questionnaire was adapted, and 328 farmers were recruited. Data were analyzed using descriptive statistics, the Chi-Square test, and Fisher's exact test.

Results: About 71.0% of the farmers were males, with a mean age of 53.04 ± 11.72 years old. Farmers had the lowest knowledge of pesticide container disposal (44.5%), pesticide mixing as a label recommendation (50.3%), and the entry route of pesticides (51.8%). The lowest awareness scores were for using personal protective equipment (PPE) while applying pesticides (23.5%), followed by wearing PPE while applying pesticides (64.6%) and washing spray tanks in a river or waterway (82.0%). Changing clothing immediately after pesticide application received the lowest score (6.7%), followed by wearing goggles during pesticide application (14.9%) and showering soon after pesticide application (15.5%). The farmers complained that they had experienced symptoms such as headaches (29.6%), fatigue (20.4%), blurred vision (19.8%), eye irritation (18.3%), and dizziness (14.6%). Farmers who mixed pesticides with their bare hands reported a higher prevalence of headaches (OR = 0.56, 95% CI = 0.33-0.92), itchy (OR = 0.37, 95% CI = 0.19-0.71), and blurred vision (OR = 2.22, 95% CI = 1.11-4.47).

Conclusion: Farmers' health-prevention practices need to be improved, as well as their knowledge and awareness. As a result, farmers must receive continuous pesticide safety education, and also training in the use of PPE during and after pesticide application.

Keywords: *pesticide exposure; pesticide application; adverse health effects*



The Effect of Combined the Pursed Lips and Lower Costal Breathing Exercise and Chest Mobilization Program on Lung Volume for Acute Exacerbations of Chronic Obstructive Pulmonary Disease.

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ABSTRACT

Introduction: Changes of the structure of the lungs and bronchi in patients with chronic obstructive pulmonary disease and acute exacerbations lead to increased airway resistance and expiratory flow limitation (EFL). This results in increase end expiratory lung volume (EELV) or air trapping in the alveoli. The patient has more symptoms including shortness of breath, cough, and sputum production.

Objective: The aim of this study was to investigate the pursed lips and lower costal breathing exercise and chest mobilization on lung volume and dyspnea in patients with chronic obstructive pulmonary disease after acute exacerbations (AECOPD) and hospitalization.

Methods: Ten patients (7 males; mean age 72 years) diagnosed with AECOPD, who had stable of vital sign (such as SBP between 30-140 mm Hg, DBP between 60-90 mm Hg, SpO₂ > 90%), good cooperation and communication, received the pursed lips and lower costal breathing exercise and stretch anterior chest wall program. Each exercise was performed 10 times/set, 3 sets/day for 2 day consecutively. The slow vital capacity (SVC) and dyspnea were measured before and after treatment period.

Results: The result indicated that the usual care program showed significant improvement in SVC after 2 days of treatment (p=0.03). However, the rating perceived exertion (RPE), heart rate (HR) and pulse oxygen saturation (SpO₂) showed no significant change (p=0.591, p=0.604 and 0.193 respectively).

Conclusion: The results suggest that the pursed lips and lower costal breathing exercise and stretch anterior chest wall program were effective for increasing the SVC and safe among the chronic obstructive pulmonary disease with acute exacerbations during hospitalization.

Keywords: COPD with AE; pulmonary rehabilitation; dyspnea



Abstract

Open Access

Development of Alcohol Behavioral Assessment Tool for Hill Tribe Adolescents: Validity and Reliability of the Tool

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ABSTRACT

Introduction: Drinking alcohol is a significant health behavior that should be changed. Hill tribe adolescents face a significant increase in the number of new drinkers. Changes in alcohol drinking behavior among hill tribe adolescents must be carried out according to the factors measured by quality measuring tools.

Objective: This research aims to study the validity and reliability of an alcohol behavioral assessment tool for hill tribe adolescents to help evaluate their drinking alcohol behavior.

Methods: Mixed Methods are divided into qualitative and quantitative research. In-depth interviewers collected the hermeneutic phenomenology data. The Lahu tribe village and the informants were selected for convenience sampling. They are three drinking and three non-drinking Lahu tribe adolescents, one community leader, two health promotion hospital staff, two village health volunteers, two youth parents, and two youth friends. The data was analyzed by Leonard's method and applied to PRECEDE MODEL. Five experts validated the content validity based on the index of item-objective congruence (IOC). In terms of internal consistency reliability, it was calculated by Cronbach's alpha coefficient from the experimental survey of 30 hill tribe adolescents.

Results: The assessment tool was divided into 6 parts: 1) Population characteristics questionnaire, 2) Alcohol drinking behavior interview form, 3) Predisposing factors in alcohol knowledge questionnaire, 4) Predisposing factors in beliefs about drinking alcohol questionnaire, 5) Predisposing factors in values about drinking alcohol questionnaire, and 6) Reinforcing factors and enabling factors questionnaire. The quality of the tool in terms of content validity, IOC of edited parts 1 - 5 was 0.81 - 1.00. Regarding reliability, Cronbach's Alpha Coefficient of edited parts 3-5 were 0.70, 0.78, and 0.70, respectively.

Conclusion: The alcohol behavioral assessment tool for hill tribe adolescents was consistent with the content validity and reliability according to the specified criteria. It will allow researchers to accurately assess drinking behavior and use the information to plan further health behavior change programs.

Keywords: *assessing alcohol behavioral; hill tribe adolescents*



Proliferation and Survival Enhancing Effect of Pinocembrin on Immortal Human Epidermal Cells

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ABSTRACT

Introduction: Pinocembrin is one of the active compounds that is found in many plants, mostly in the Zingiberaceae family. Previous studies reported that pinocembrin possesses wound healing enhancing activities. However, the wound healing-promoting activities of pinocembrin in human cell lines have not been fully elucidated; especially for the aspect associated with the growth and survival signaling pathways.

Objective: To investigate possible molecular mechanisms of pinocembrin in enhancing growth and survival signaling pathways in human keratinocytes

Methods: Immortal human keratinocyte cell line (HaCaT) was used as an in vitro model. Western Blot analysis and immunofluorescence techniques were used to investigate the molecular mechanisms of pinocembrin. Specific inhibitors of proliferation and survival signaling cascades, which were U0126 and LY294002, were also used for confirming the mechanism of pinocembrin.

Results: Pinocembrin activated ERK1/2 and Akt phosphorylation which play important roles in growth and survival signaling pathways, respectively. The effects were seen in dose-dependent fashion. Moreover, MEK inhibitor (U0126) and PI3K inhibitor (LY294002) confirmed that pinocembrin induces proliferation and survival through stimulating the MAPK and PI3K/Akt signaling pathways.

Conclusion: Pinocembrin induces the proliferation and survival of human keratinocyte cells. Its mode of action is mediated through the activation of ERK1/2 and Akt which typically function in maintaining cellular proliferation and survival. In summary, the current study supports the wound healing-enhancing effect of pinocembrin at the molecular level and further supports its potential therapeutic applications for skin regeneration.

Keywords: *pinocembrin; flavonoid; keratinocytes; regenerative medicine*



The Anti-cancer Effects of Oxyresveratrol on Ovarian Cancer Cell Lines via Negative Regulation of AKT Activation Status

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ABSTRACT

Introduction: Oxyresveratrol has been reported that inhibits the survival signaling and has anti-cancer effects on various cancers. However, the difference in the cancer genotype typically influences the responsiveness to anti-cancer agents. Therefore, comparative observation for the response sensitivity to Oxyresveratrol should be determined. Oxyresveratrol was tested in different ovarian cancer cell types for its cytotoxic effects, the expression level of pro-apoptotic and anti-apoptotic proteins, and the inhibitory influence on survival signaling pathway.

Objective: To investigate the anti-cancer effects of oxyresveratrol on different types of ovarian cancers and identify the possible mechanisms of oxyresveratrol.

Methods: Oxyresveratrol was tested in three different ovarian cancer cell lines, A2780, SKOV3, and TOV21G. Cytotoxic effect was evaluated by MTT, and western blot was used to determine the level of pro-apoptotic proteins, anti-apoptotic proteins, and key molecules in survival signaling transduction. Cisplatin was used as a standard chemotherapy to determine the efficacy of the combinational treatment.

Results: Oxyresveratrol induced cell apoptosis in A2780 and SKOV3, reduced anti-apoptotic protein levels, and activated the expression of pro-apoptotic proteins. Moreover, oxyresveratrol inhibited phosphorylation of AKT and PDK-1 without any effect on the phosphorylation status of ERK1/2. In combination with cisplatin, oxyresveratrol amplified the efficacy of the drug to induce cell death in SKOV3, generally resistant to cisplatin.

Conclusion: Oxyresveratrol inhibits cancer cell apoptosis via reduction of PI3K-AKT transduction, and that decreases the level of anti-apoptotic proteins and activates cell apoptosis. Moreover, oxyresveratrol combined with a standard chemotherapy can amplify the death-inducing efficacy in resistant cells.

Keywords: *oxyresveratrol; ovarian cancer; anti-cancer effects*

Discriminative Ability of the 7th Cervical Vertebral Wall Distance for Hyperkyphosis Measurement in Elderly

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ABSTRACT

Introduction: Hyperkyphosis is an excessive thoracic spine curvature exceeding 40 degrees, which can lead to many adverse health and other health-related consequences. Currently, the 7th cervical vertebra wall distance (C7WD) is a practical, valid, and reliable measure to screen and monitor thoracic hyperkyphosis in the elderly. The C7WD is at least 7.5 cm. could indicate the risk of hyperkyphosis in elderly. However, there is no clear evidence to confirm the clinical utility of the measurement as a discriminative ability of the C7WD.

Objective: To investigate discriminative ability of hyperkyphosis severity using C7WD in the elderly

Methods: This study was conducted in 90 participants, aged at least 60 years from several communities who had different degrees of thoracic hyperkyphosis. All participants were assessed for their severity of thoracic hyperkyphosis using the C7WD and a lateral plain radiograph (Cobb's method). Hyperkyphotic elderly was defined using Cobb angle of at least 40 degrees and C7WD of at least 7.5 cm.

Results: Participants were divided into 2 groups including normal group (C7WD less than 7.5 cm and hyperkyphosis group (C7WD at least 7.5 cm). The finding showed the significance difference of C7WD and Cobb angle between the normal (C7WD 3.57±2.59 cm, and Cobb angle 32.84±4.54) and hyperkyphosis group (C7WD 9.26±3.05 cm, and Cobb angle 46.13±5.15) (p<0.001).

Conclusion: The finding suggested that the C7WD was a simple method for hyperkyphosis screening in which elderly with the C7WD at least 7.5 cm may be an initial hyperkyphosis.

Keywords: Hyperkyphosis; Dowager's hump; discriminative ability; spinal angle; 7th cervical vertebra wall distance



Comparison of Executive Function in Male Puberty and Andropause

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ABSTRACT

Introduction: Andropause or male menopause is associated with a decrease in executive function, activity, daily living performance, and quality of life. However, no study reported the executive function skill in andropause or male menopause.

Objective: To compare the executive function in male puberty and andropause in five skills, including mental flexibility, inhibitory control, verbal fluency, working memory, and planning.

Methods: 18 male puberty and 13 andropause underwent executive function test including mental flexibility by D-KEFS sorting test, verbal fluency by verbal fluency test, inhibitory control by Stroop test, working memory by trail making test part B and planning by D-KEFS tower test. Statistical analysis by Independent-sample t-test in normal distribution and Mann-Whitney U test in non-normal distribution and p-value is 0.05.

Results: In the male puberty group average age is 20.17 ± 0.79 years old, and in the andropause group, the average age is 48.54 ± 5.52 years old. This study found that there are 3 tests that showed significant statistical differences ($P < 0.05$) between groups, including mental flexibility ($p = 0.002$), verbal fluency ($P < 0.001$), and inhibitory control ($P < 0.001$). However, there are two tests that showed no significant statistical difference, including working memory ($P = 0.068$) and planning ($P = 0.170$).

Conclusion: Executive function, including mental flexibility, verbal fluency, and inhibitory control in andropause, was significantly less than the male puberty group. Therefore, executive functioning skills in andropause should be concerned with preparing for aging.

Keywords: executive function; andropause; male puberty; Delis-Kaplan executive function system (D-KEFS)

The Immediate Effects of Star Excursion Balance Training on Balance and Walking Ability in Healthy Elderly People: A Randomized Controlled Trial

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ABSTRACT

Introduction: Aging is defined as a natural process characterized by structural and functional changes. Functional impairments are linked to changes in balance and walking ability with aging. These impairments are the most common cause of falls among the elderly, which also reduce their quality of life. Therefore, balance training is essential for fall prevention in elderly people.

Objective: To study the immediate effect of star excursion balance training on balance and walking speed in healthy elderly people.

Methods: Thirty-eight participants were aged 60 to 69 years old, both males and females. All participants were randomized into 2 groups including the experiment group that received star excursion balance training (n=19) and the control group that received stretching exercise (n=19). Both groups received 10 minutes of warm-up and cool-down and 30 minutes of exercise per session for 1 session. Static balance control was assessed by measuring the single-leg stance (SLS) test, dynamic balance control was assessed by measuring the time up and go (TUG) test, and walking speed was assessed by measuring the 10-meter walk test (10MWT), respectively. All outcome measurements were tested before and after receiving the intervention.

Results: The results revealed that there were no significant differences in all outcome measurements between groups (p>0.05). In comparison within groups, there were no significant differences in the SLS test in both groups (p>0.05). However, there were significant differences in the TUG test and 10MWT after receiving intervention within the experimental group (p<0.05).

Conclusion: The immediate effects of star excursion balance training resulted in improved dynamic balance and walking speed. The results of this study will provide useful clinical information for future rehabilitation programs for the elderly. However, this study focused on the immediate effect of star excursion balance training. This may not cover the long-term effects of exercise on balance in the elderly.

Keywords: *balance; star excursion balance training; elderly; walking speed; randomized controlled trial*



The Short Time Effect of Kinesio Taping on Back Muscle Endurance, Lower Back Flexibility and Balance in Sedentary Young Adult

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ABSTRACT

Background: A sedentary lifestyle was often experience problems with the musculoskeletal system. Less physical activity will reduce the capability of the muscles. Moreover, it can cause pain or diseases related to the musculoskeletal system. Therefore, this study aimed to examine the effect of Kinesio taping on back muscle endurance, lower back flexibility and balance in sedentary young adults.

Method: This study was a randomized controlled trial, 44 sedentary young adults were selected and subjects were randomly assigned into two groups, the KT group and the control group. Each group included 22 subjects, they were assessed before the taping intervention of Schober's test, Single stance test, Y-balance test and Biering-Sorensen test. After the taping intervention 30 minute, subjects were recorded using the same test method.

Result: There was a statistically significant increase of single leg stance in the KT group compared to the control group ($p \leq 0.05$). In other tests, the KT group was significantly increased compared to before and after the experiment. On the other hand, the flexibility of the back had a statistically significant decrease in the KT group. Besides, there was no significant difference of the flexibility of back between in the KT group and the control group.

Conclusion: In this study found that the kinesio tape can increase capability of balance in people with sedentary behavior with statistical significance. Due to kinesio tape can increase the sensory response in the area where the tape is attached. This result shows Kinesio taping seems to have an effect on perception and the ability of the nervous system and muscles to control movement.

Keywords: *Kinesio tape; endurance; Balance; Sedentary lifestyle*

Comparison Physiological Effect and Comfortable Sensation between Wearing Surgical Face Mask and Muslin Face Mask during Exercise in Healthy Subject: A Pilot Study

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ABSTRACT

Introduction: Widely spread of COVID-19 virus worldwide leads to changes in daily living situations such as; keeping social distance, frequently cleaning hands, and wearing a face mask all the time even while exercising to against the spread of the virus. **Objective:** This study aims to compare the effect of wearing 3 types of face masks; Non face masks (NFM), Surgical face masks (SFM), and Muslin Face masks (MSM); while exercising with moderate intensity to cardiovascular response and comfortable feeling while wearing face mask in healthy adult participants.

Methods: Cross-over design was used to investigate the study's objective. Ten healthy participants aged 20.9±1.5 years old, BMI of 21.26±1.33 kg/m² performed treadmill exercise while wearing NFM, SFM, and MFM with an intensity of 40-59% Heart rate reserve (HRR) for 30 minutes. Perceived Comfort Scale (PCS) and Cardiovascular response including; Blood pressure (BP), Respiratory rate (RR), Oxygen Saturation (O₂sat), Rating Perceived Breathlessness (RPB), and Borg Rating of Perceived Exertion (RPE) were recorded in each exercise session with a wash-out period for 48 hours.

Results: All participants can complete the exercise with moderate intensity while wearing 3 types of face masks. After 3 sessions of exercise, systolic BP and PCS showed a significant difference between types of face masks (p-value = 0.024, 0.033, respectively). Change SBP between baseline to exercise have significant difference when compare NFM vs SFM (p-value = 0.042) and NFM vs MFM (p-value = 0.035); Change SBP value equal 9.3±11.52 mmHg in NFM, 18.8±7.38 mmHg in SFM, and 18.9±6.87 mmHg in MFM. PCS scores have a significant difference in NFM vs MFM; score 25.9±2.02 and 28.5±3.41, respectively (p-value = 0.018). Diastolic BP, RR, O₂sat, RPB, and RPE were not found significantly different between groups.

Conclusion: Wearing SFM and MFM while exercising with moderate intensity leads to increased SBP and discomfort feeling more than NFM. However, it seems to be that wearing SFM and MFM does not affect exercise performance and dangerous responses for healthy adults. According to this pilot study, further studies with a larger sample should confirm these initial results.

Keywords: *surgical mask; muslin face mask; treadmill exercise; aerobic exercise; comfortable sensation*



Antimicrobial Activities of *Streblus Asper* Leaves Extract in Thailand

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ABSTRACT

Introduction: *Streblus asper*, or toothbrush tree, has been traditionally used as a medicinal plant for toothache and dental caries treatments.

Objective: This study aimed to investigate antimicrobial activities of *S. asper* leaves ethanolic extracts in Thailand.

Methods: *S. asper* leaves were washed, dried and ground to powdered, then exhaustively extracted with 95% ethanol using Soxhlet extraction. Antimicrobial activity including determined zone of inhibition, determination of minimum inhibitory concentration (MIC), determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined, as according to Clinical & Laboratory Standards Institute guidelines. The extract was done against 10 tested microorganisms including, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus mutan*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida glabrata*.

Results: *S. asper* leaves extract at 200 mg/ml showed an inhibition zone against tested microorganisms, except *Pseudomonas aeruginosa*, ranging from 7.00 to 11.00 mm. The leaves extract had inhibitory and microbicidal capacity at concentration of $\geq 2,000$ $\mu\text{g/ml}$, except *Staphylococcus aureus* and *Streptococcus mutan* had good inhibitory and microbicidal capacity at concentration of 125 and 250 $\mu\text{g/ml}$, respectively.

Conclusion: *S. asper* leaves extract had good activities on oral pathogen bacteria, its leaves extract could be beneficial as an oral hygiene product.

Keywords: *Streblus asper*; clear zone; MIC; MBC; MFC

Full proceeding

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**Full proceeding
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Analysis of the clinical therapeutic effect of Professor Wang Yue on 42 cases of Sjogren's syndrome overlaps with Hashimoto's thyroiditis

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ABSTRACT

Introduction: Clinical work and reports in the past ten years have found abnormal thyroid function in many patients with Sjögren's syndrome (SS), and Hashimoto's thyroiditis (HT) is the most common. Both are Autoimmune diseases, and there is currently no effective treatment. Traditional Chinese Medicine can effectively improve patients' clinical symptoms and laboratory indicators through syndrome differentiation, treatment, and holism modulating.

Objective: To examine the TCM method of differentiation, treatment, medication characteristic, and clinical effect of Professor Wang Yue in treating primary Sjogren's syndrome overlap with Hashimoto's thyroiditis (SS-HT).

Methods: 42 cases with SS-HT from the outpatient department of Professor Wang Yue of Nanjing Affiliated Hospital of traditional Chinese medicine between January 2015 to August 2020 were selected. Excel 2010 was used to collect and frequency analysis the general data, syndrome types, and medication characteristics of Professor Wang Yue in treating SS-HT, and used SPSS 24.0 software to statistically analyzed the TCM syndrome score and laboratory index before and after treatment.

Results: Professor Wang Yue mainly treated SS-HT by replenishing Qi and nourishing Yin, generating fluid and relieving thirst, clearing heat and purging fire, reducing swelling, and dispersing knots. Six cases were markedly effective, 15 cases were improved, 19 were effective, and two were ineffective. The total efficiency was 95.24%. After treatment, TCM syndrome score decreased significantly ($P < 0.01$), CRP, ESR, TGA, TPOAb decreased significantly ($P < 0.01$).

Conclusion: SS-HT's pathogenesis is Yin deficiency and internal heat in the first stage. In the middle stage is both Qi and Yin deficiency, and liver and kidney Yin deficiency are in the late stage. SS-HT treatment method moistens the lung and clears the stomach heat, replenishing Qi and Yin, tonifying the liver and kidney, and reducing swelling and dispersing knots. Chinese Medicine treatment of SS-HT has a definite curative effect: it can relieve clinical symptoms and reduce the inflammatory index and thyroid autoantibody without side effects, including anti-inflammatory, analgesic, anticoagulation, immune regulation, liver, and kidney protection. It is worthy of clinical application.

Keywords: Sjögren syndrome; Hashimoto's thyroiditis; TCM syndrome differentiation; autoimmunity

Introduction

近十年在临床工作和文献报道中发现很多干燥综合征 (Sjögren's syndrome, SS) 患者常伴有甲状腺功能异常, 其中桥本氏甲状腺炎 (Hashimoto's thyroiditis, HT) 为多见。报道显示 HT 在 SS 患者

的发病率为正常人群的 9 倍, 而 pSS 患者中引起甲状腺疾病的主要病因是 HT [1]。中医在辨证论治的基础上治疗自身免疫性疾病具有优势, 与西药联合应有不但能提高疗效, 还能减少西药的副作用, 改

善患者的生活质量。但目前针对 SS 合并 HT 的相关文献及研究较少，只限在病案报道。

汪悦教授是江苏省名中医，南京中医药大学博士研究生导师，在临床实践 30 多年，对治疗 SS-HT 有丰富的临床经验。故本研究目的为探讨分析汪悦教授辨治 SS-HT 临床疗效，安全性及用药经验，助于中医治疗逐步走向规范化，对临床治疗及预防结缔组织病合并甲状腺疾病提供一定的启示。

Methodology

资料与方法

1. 研究对象

选取病例资料为 2015 年 1 月至 2020 年 8 月期间在江苏省中医院汪悦教授门诊 SS-HT 患者 42 例。

2. 诊断标准

2.1 西医诊断：SS 参照 2002 年干燥综合征国际分类标准 [2]，HT 参照《中国甲状腺疾病诊治指南》标准 [3]。

2.2 中医诊断：参考《中药新药临床研究指导原则(试行)》风湿免疫系统病和内分泌代谢系统疾病的相关证型的标准，并结合 SS 和 HT 的自身特点，拟定如下：①阴虚内热：主症：口眼干燥，颈前不适或肿大，盗汗，五心烦热；次症：心悸，失眠；舌脉：舌红少苔，脉细弦。②气阴两虚：主症：口干目涩，颈前不适或肿大，关节隐痛，乏力；次症：自汗盗汗，耳鸣，眠差多梦，大便干结；舌脉：舌红或淡红，苔花剥或薄白，脉细或细数。③气血两虚：主症：口干目涩，视物模糊，颈前不适或肿大，面色苍白发，神疲乏力，汗出，心悸气短。次症：眩晕耳鸣，月经量少色淡或闭经；舌脉：舌淡苔薄，脉细无力。④肝肾阴虚：主症：口干目涩，视物模糊，颈前不适，关节酸痛，腰膝酸软。次症：乏力，脱发，眩晕耳鸣，或有低热，月经不调；舌脉：舌质红，苔少或剥脱，脉沉细弦。⑤肾阳亏虚：主症：口干目涩，颈前肿块，腰膝酸软，怕冷，手足不温；次症：关节隐痛，寐差，大便稀溏；舌脉：舌质淡，苔白，脉沉细。⑥痰凝血瘀：主症：颈前结块肿大，质韧或硬、时刺痛、固定不移、渴不欲饮、咯痰不爽，口干目涩，关节肿大疼痛或刺痛，关节畸形；次症：面色萎黄、胸闷、纳呆；舌脉：舌暗或有斑点或紫、苔白腻或薄白，脉滑或涩或弦。

3. 纳入标准和排除标准

3.1 纳入标准：1) 年龄≥18 周岁；2) 符合诊断标准者；3) 中药内服为主要治疗方法；4) 基本方药物、药量记载完整；5) 有复诊和复查记录。

3.2 排除标准：1) 合并恶性肿瘤患者；2) 用过影响甲状腺功能的药物或既往有甲状腺手术史；3) 妊娠期或哺乳期妇女；4) 临床检查资料不完整者。

4. 研究方法

4.1 利用 Excel 2010 收集患者的一般资料，诊断，中医证型，方药；

4.2 观察患者在治疗前后的相关实验室指标及中医证候积分并进行比较，疗程 3 个月为一个疗程。

5. 观察内容

5.1 安全性指标：C 反应蛋白 (CRP)，血沉 (ESR)，甲状腺自身抗体 (TGAb、TPOAb)；不良反应：观察肝、肾功能。

5.2 中医证候积分：参照《中药新药临床研究指导原则(试行)》，各型患者症状中凡具备主症≥2 项及次症≥1 项者，结合舌脉即可做出诊断。根据严重程度，主症为分别为：0、2、4、6；次症分别为为 0、1、2、3。计算公式 (尼莫地平法) 为：[(治疗前积分-治疗后积分)÷治疗前积分] ×100%。

5.3 总体疗效评定：本研究疗效评定标准疗效分为显效、进步、有效和无效。显效：中医证候积分减少≥75%，ESR 及 CRP 正常或明显改善；进步：中医证候积分率≥50%，ESR 及 CRP 正常或有改善；有效：中医证候积分率≥30%，ESR 及 CRP 有所改善或无改善。或中医证候积分减少 30%-100%，ESR 及 CRP 无改善；无效：中医证候积分率<30%。ESR 及 CRP 无改善。

6. 数据统计分析

6.1 用 Excel2010 软件进行统计与归纳 SS-HT 患者的用药次数及频率。

6.2 采用 SPSS 24.0 软件进行统计分析治疗前后的中医证候积分及实验室指标比较。计量资料的治疗前与后比较，如结果符合正态分布均采用配对 t 检验，若不符合正态分布的采用 Wilcoxon 符号秩检验进行比较。

Results

1. 一般资料

SS 合并 HT 共 42 例，女性患者 41 例 (97.62%)，男性患者 1 例 (2.38%)，平均年龄为 45.38±10.36。

2. 中医证型分布

最常见为阴虚内热证 20 例 (47.61%)，其次为气阴两虚证 18 例 (42.86%)，气血两虚证和肝肾阴虚各 2 例 (9.53%)。

3. 用药规律

3.1 高频用药分析：

所用 111 味中药，药物使用共 608 次，使用频次前十六味中药见表 1。治疗 SS-HT 最多使用的中药为

生地黄，使用频率占每张处方的 95.24%。其中使用频率超过 50% 的中药还有麦冬、石斛、金银花、白芍、甘草。

表 1 SS-HT 患者常用药

药物	频次	频率	药物	频次	频率
生地黄	40	95.24%	土茯苓	17	40.48%
麦冬	33	78.57%	五味子	16	38.10%
石斛	33	78.57%	玄参	15	35.71%
金银花	32	76.19%	黄芩	14	33.33%
白芍	31	73.81%	芦根	12	28.57%
甘草	31	73.81%	夏枯草	12	28.57%
黄芪	20	47.62%	连翘	12	28.57%
天花粉	18	42.86%	乌梅	11	26.19%

3.2 中药性味归经分析：

使用药性为性寒和微寒，药味以甘为主，归经多为肺及胃经为主，其次为脾，肝，心和肾经。

3.3 中医功效及药理作用分析：

所得结果提示治疗 SS-HT 的中医功效主要以益气养阴，生津止渴和清热泻火解毒，清肝明目，消肿散结。病例前十六味用药的药理作用的分析结果表明，治疗 SS-HT 的药理作用对免疫力功能作用最多（增强免疫，调节免疫，免疫抑制），其次为抑菌，降血糖，保肝，抗氧化，抗炎，保护心脑血管等作用。

4. 疗效分析：

总体疗效评价，治疗后显效 6 例，进步 15 例，有效 19 例，无效 2 例，总有效率为 95.24%。治疗后中医证候积分较前明显降低，差异具有统计学意义 ($P < 0.01$)。治疗后 CRP, ESR 改善均具有显著的统计学意义 ($P < 0.01$)；治疗后 TGAb、TPOAb 较前降低，差别具有显著的统计学意义 ($P < 0.01$) 见表 2。安全性指标上治疗后患者未出现肝肾功能异常。

表 2 SS-HT 治疗前后比较

	治疗前	治疗后	P
中医证候积分	10.71±3.47	4.86±2.42 ^{▲▲}	0.000
CRP	4.80±4.57	3.52±2.71 ^{▲▲}	0.007
ESR	29.28±24.08	16.86±12.24 [▲]	0.000
TGAb	416.80±415.2	346.7±399.0 [▲]	0.000
TPOAb	432.00±425.6	282.80±327.90 ^{▲▲}	0.000

*注:与治疗前相比,▲表示 $P < 0.05$; ▲▲表示 $P < 0.01$ 。

Discussion

1. SS-HT 病因病机

SS 的特点主要因阴津亏虚为主，临床表现多为，眼目干涩，口燥咽干，皮肤干燥，口渴欲饮等燥热之症，而 HT 的临床表现多为颈前肿大或不适感，疲劳乏力，汗多，怕冷等阳虚痰凝之象。临床上 SS 合并 HT 的发病多数患者以 SS 表现较典型，可伴有一些 HT 的特征但不明显。汪悦教授认为 SS-HT 的临床表现多为本虚表实，从审症求因的传统认识出发，若燥热之象较为明显者，可归属于“燥证”的范畴。若 HT 的症状较明显则归为“瘰病，虚劳”的范畴。

临床研究发现甲状腺疾病可在结缔组织病发生前或者后阶段。本次研究表明本病发生在中年女性为多，因女子以肝为先天，中年女子，肝肾阴虚，天癸渐竭，精血亏虚，血不养肝，加上女子多有经产乳育，易耗伤阴血，导致血虚肝旺，燥热内生，则出现 SS 的阴虚内热之象。久则肝木克脾土，导致脾失健运。脾为生痰之源，脾失健运，水湿内停，日久聚而为痰，阻滞气机，气滞血瘀，结于颈前；或情志失调，肝失条达，气机郁滞，津凝痰聚，久则成瘰，结于颈前，皆可发展为 HT。瘰病主要病机为气郁痰凝，痰气搏结颈前，日久导致血脉瘀阻。疾病早期多为实，病变日久，气郁化火，伤津耗气，导致肺脾气虚，津液失布，机体筋脉九窍失于濡养，则出现口燥眼干，皮肤干燥，阴道干涩等 SS 之症。汪师认为本虚为本病之根本，禀赋不足、劳倦过度、情志内伤、年高体弱、久病失养、误治、失治、六淫外邪是致病因素，而“痰”和“瘰”为本病发生过程有为病理产物。其病位主要在肝，脾，肺，胃，肾脏。

2. SS-HT 用药分析

SS-HT 患者临床表现多以燥热之象为明显，据“燥者润之”，用药多以“甘寒滋润”，涉及的脏腑为肺脾胃肝肾。根据常用药物的性味归经及功效，提示汪悦教授治疗 SS-HT 用药注重润肺清胃，益气生津，重视养肝柔肝，以达到生津润燥之目的。汪师把本病分为早中晚三期来论治。

2.1 润肺燥，清胃火，兼顾脾胃

本病初期以阴虚内热为主，其临床表现为口干咽燥，皮肤干燥，心烦口渴，五心烦热，心悸，关节肌肉隐痛，失眠，月经过少或不调，便秘，舌红少苔，脉细数或弦。HT 的症状并不显。其治法以滋阴清热，肺胃兼顾。清肺热，润肺燥，滋养肺胃之阴的同时，常配益气健脾之药物，一方面是防止燥邪耗伤气阴，另一方面也助脾运化，使津液的布散功能恢复正常，避免痰，瘰等病理产物生成而加重 HT，固其本。导师认为肺气的宣发和肃降对体内的水液代谢起着重要的作用，“上焦如雾”，再如张景岳说“阴虚者，要补而兼清”。因此治疗药

性以轻清，清中有补，润燥生津而不敛邪，标本兼顾。常用方药为清燥救肺汤，增液汤，沙参麦冬汤，益胃汤，百合固金汤等。药为生地黄，麦冬，玄参，沙参，金银花，石斛，五味子，白芍，甘草，黄芩等。在滋阴清热的基础上，因治疗本病的药物多为甘寒滋腻易碍胃，苦寒败胃，则常配伍性温的苍术和半夏燥湿健脾，顾护胃气；另外玄参和半夏有散结之功，有宜于治疗 HT。眼干较甚者加枸杞子，谷精草，密蒙花，五味子；口干较甚者加天花粉，芦根，乌梅；关节疼痛者加桂枝，秦艽，防风藤；颈前肿块者可加夏枯草，连翘，山慈菇，浙贝母；低热，五心烦热者加知母。大便干结者加熟地黄，当归，桃仁；兼有血瘀者可加丹参，丹皮。

2.2 益气养阴，消肿散结

在疾病中期阴虚内燥日久，伤津耗气，导致肺脾气虚，脾失健运，肺失宣发，痰湿内生，痹阻经络，而兼有痰瘀互结之症状，而 SS 和 HT 的症状两者较显。临床患者口眼干燥，颈前结块或不舒适明显，伴有少气懒言、疲劳乏力，自汗盗汗，关节隐痛，寐差，便秘，月经不调，舌淡红或淡白、苔花剥或薄白，脉细或细数等气阴两虚之症。治当益气养阴，兼以化痰散结。方用增液汤，参苓白术散，补中益气汤等，药为太子参，黄芪，生地，麦冬，玄参，芍药，甘草等，白术，连翘，夏枯草。少气懒言者加党参，太子参，白术；痰湿较甚者加茯苓，白术，半夏，苍术；盗汗明显者加白芍，五味子，月经不调者加当归，益母草，墨旱莲。

2.3 补益肝肾，温阳通络

病延多年，气阴两伤，肝肾阴虚，阴损及阳，阳虚寒盛，血脉瘀阻，痰湿内生，临床上患者口干咽燥，视物模糊，畏寒肢冷、汗少、全身关节酸痛，腰膝酸软，疲劳乏力，脱发，眩晕耳鸣，月经不调，舌质偏红，苔白或剥苔，脉沉细弦等肝肾阴虚兼阳虚寒凝之证，在后期则 HT 的症状较 SS 明显，临床常见 HT 伴发甲减。治当补益肝肾，温阳通络，兼活血化瘀。常用方为杞菊地黄丸，独活寄生汤，左归丸。药如生地黄，熟地黄，独活，桑寄生，秦艽，桂枝，杜仲，防风，白芍，淫羊藿等。腰膝酸软者加怀牛膝，狗脊，续断；怕冷，四肢不温者加附子，肉桂，细辛，淫羊藿；大便干结加桃仁，当归，肉苁蓉；下肢浮肿者加真武汤，苍桂术甘汤；偏肾阳亏虚则用金匮肾气丸和右归丸。由高频药物统计结果显示，治疗 SS-HT 药物归类多为补阴药和清热药，主要以益气养阴，生津止渴，清热解毒，清肝明目，消肿散结。现代药理学表明，80%的补阴药含有多糖或糖复合物成分，具有免疫调节，保护细胞本身，保肝，抗疲劳等作用 [4]。而清热药具有抗炎，抗菌，解热，影响心血管系统，利尿等作用 [5]。常用药如，生地黄能抑制大剂量甲状腺素诱导的 β -肾上腺素受体兴奋、增强体

液免疫和细胞免疫功能 [6]、护肝、抗骨质疏松、雌激素样等作用 [7]；白芍有抗炎镇痛、抗抑郁、抗癌、抗血栓、降血脂、降糖、保肝、通便等作用 [8]；麦冬有免疫调节、抗炎、心血管保护作用、降血糖、降血脂等作用 [9]；石斛具有免疫调节、抗疲劳、抗氧化、抗肿瘤、降尿酸等作用 [10]；金银花具有抗炎、护肝、增强免疫、抗凝等作用 [11]。

Conclusion

研究表明，中医药在治疗 SS-HT 疗效肯定，具有良好的安全性，适合患者长期坚持治疗。使用养阴清热法治疗 SS-HT，可改善患者的临床症状及实验室指标。通过分期治疗，初期以润肺燥，清胃火，兼顾脾胃，中期以益气养阴，消肿散结，后期以补益肝肾，温阳通络，并以辨证与辨病结合，标本兼顾，可有效的缓解临床症状、降低患者的炎症指标 (CRP, ESR) 以及甲状腺自身抗体 (TGAb, TPOAb)，并无副作用。其具体可能起到抗炎，止痛，免疫调节，抗疲劳，抗凝，保护肝肾等作用。

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The History and Development of TCM Ointment Therapy

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ABSTRACT

Ointment massage (paste massage) is a traditional external treatment method of traditional Chinese medicine. It is a form of massage and ointment used together by using the ointment as the medium, applying paste before manipulation, and then applying the Tuina massage technique. The ointment has been used for over 2000 years, as early as the pre-Qin period. With the development of history, from the northern and Southern Dynasties, Sui and Tang Dynasties, Song, Jin, Yuan Dynasties, and Ming and Qing Dynasties. Up to now, ointment massage therapy can treat various clinical diseases and has a significant curative effect, which more and more patients have recognized. This paper showed the development of ointment in Traditional Chinese Medicine history to the modern period.

Keywords: ointment massage therapy; the history of TCM ointment therapy; TCM external therapy; paste massage

1. Origin of Ointment

The development of ointment massage therapy in China has a long history, recorded in many historical pieces of literature on traditional Chinese medicine. The earliest record of ointment massage therapy is *52 Bing Fang (Prescriptions for fifty-two diseases)*. The content of ointment massage therapy was first recorded in *Huangdi Neijing (The Yellow Emperor's Canon of Internal Classic)*. There are also records of medicinal rubs or ointments, mainly used to treat dermatoses, such as itch of skin and frostbite. However, the prescription composition is not recorded [1-3].

2. The emergence of the term "Ointment"

The classic literature *Synopsis of prescriptions of the Golden Chamber* put forward the word "ointment massage." Ointment massage has become an external treatment combining massage techniques and drugs, which has attracted the attention of doctors of all dynasties. This literature puts forward the concept of preventing and treating diseases. "The way of promoting health preventing diseases. As soon as the evil Qi

reaches the meridians and collaterals, it should be treated immediately, not waiting for it to go deep into the viscera. In case of serious pain and numbness in the limbs, the channels and collaterals are blocked. We should use methods such as guidance, exhalation, acupuncture, ointment external application, and massage so that the meridians and orifices are not blocked. The five flavors of diet should be balanced, not partial to food. Excessive sexual activity can exhaust kidney essence. If the body's healthy Qi is strong, the evil Qi will not invade the internal organs and meridians and get sick. The *Synopsis of prescriptions of the Golden Chamber* also records the ointment massage prescription "Tou Feng Mo San Gao": Dafuzi and salt rub the rash with a square inch dagger so the drug can be effective." It has become the origin of the "Mo Ding Gao" ointment in the later period [4].

3. History and progress of ointment massage therapy

3.1 Two Jin's northern and Southern Dynasties

Two Jin's northern and Southern dynasties were the period of the formation and initial development of

ointment. The prescription of ointment has been scattered in the preserved literature. Moreover, there is a relatively complete prescription of "Gaomo (ointment)," as well as disease-specific and application methods. Become the foundation of the development of ointment massage therapy for later periods [5].

3.1.1 Huangdi Neijing (The Yellow Emperor's Canon of Internal Classic) and ointment

According to Huangdi Neijing, Ointment massage therapy is clinically used to treat facial paralysis and other diseases. *Lingshu Jingjin Chapter 13* records, "The treatment for the disease of Foot Yangming's meridians is to use horse ointment, stick it on the side of the contracture and convulsion. Then mix cinnamon powder with Baijiu (distilled spirits), apply it on the loose side, hook it in the corner of the mouth with a mulberry hook, and place it in a small pot with the charcoal fire of mulberry firewood. The high and low position is subject to the patient's slow breathing when sitting. While ironing the cheek on the contracture and convulsion side with horse ointment, drink some wine and eat more delicious things like bacon. People who cannot drink alcohol should also reluctantly drink some and rub the affected part repeatedly so that they can heal." This is the treatment method of ointment massage combined with massage technique for facial paralysis. In the later period, the *Sheng Ji Zonglu (General Medical Collection of Royal Benevolence)* also used Chinese honey locust ointment to treat facial paralysis. These are the fundamental theories of treating facial paralysis with horse ointment from *Lingshu Jingjin Chapter 13*, which comes from the treatment of facial paralysis with ointment in the later periods [6].

3.1.2 Zhou Hou Bei Ji Yao Fang (The Handbook of Prescriptions for Emergencies) and ointment

The ointment prescription recorded in the *Zhou Hou Bei Ji Yao Fang (The Handbook of Prescriptions for Emergencies)* can be used externally or orally. In the use of ointment massage, it is emphasized that "Xianghuomo" mean that using massage techniques to produce warmth can achieve the effect of "warming the meridians and dredging the collaterals" in order to increase the therapeutic effect. This concept has made significant progress in applying ointment massage therapy. The ointment formula has been used in medical books and modified to a certain extent in later periods. "Pei Shi Wu Du Shen Gao ointment is a prescription for treating all kinds of diseases that cause by evil pathogenic, with symptoms such as talk nonsense, dizziness, and unconsciousness: Xionghuang, Zhusha, Danggui, Chuanjiao, and Wutou, soaking in bitter wine overnight. Five jin (a unit of weight) of pig fat, fry the Chenlu five times, grind away the dregs, mix and stir with Xionghuang and Zhusha powder. Take with a

warm wine in the dosage same as jujube stone. If it does not heal, retake it until the disease gets healed; If there is a disease in the limbs, or carbuncle, swelling and other diseases and sores can be rubbed and applied with the paste. It is better to apply it to the body at night and when the disease emits fog and dew." These showed that ointment therapy and formula had been continuously used and supplemented in the clinic. The traditional Chinese Medicine drugs used in the ointment formula recorded in the above *Zhou Hou Bei Ji Yao Fang* have many indications. They can be used to treat various diseases.

3.1.3 Liu Juanzi Guiyi Fang and Gao Mo

Liu Juanzi Guiyi Fang is remarkable literature on surgery, which also records the rich records of ointment massage therapy in treating diseases. It was written in 483 years. The ointment formulas in the book include *Sheng Xiong Qiong* ointment, *Wu Du* ointment, *Sheng Rou* ointment, *Baizhi* massage ointment, *Dan Sha* ointment, *Shexiang* ointment, *Haunglian* ointment, *Ouzhi* ointment, etc., which are specialized in the treatment of surgical diseases.

3.2 Sui and Tang Dynasties

In the Sui and Tang Dynasties, plaster massage therapy got further development. Especially in the Tang Dynasty, the application of ointment massage therapy was an essential feature in the history of massage.

3.2.1 Beiji Qianjin Yaofang (Prescriptions Worth a Thousand Pieces of Gold for Emergencies) and ointment

During this period, ointment massage therapy has been extensively developed. *Beiji Qianjin Yaofang* records many ointment formulas for preventing and treating pediatric diseases. For example, *Wuwugancaoshengmo* ointment, *Mosheng* ointment, *Danshachi* ointment, *Yizhongbaiyu*, *Mifenyan*, in *Beiji Qianjin Yaofang: Shao Xiao Ying Ru Fang*. It is a lot of proven prescriptions obtained from folk experience. Sun Simiao recorded many ointment prescriptions in *Beiji Qianjin Yaofang: volume 16-Zhongfengshang Zhugaodisan, Volume 17-Feng Du Jiao Qi Gaodiwu*. There also found ointment massage therapy treating various diseases in *Beiji Qianjin Yaofang: Volume 5-Shao Xiao Ying Ru Fang, Fu Ren Yi*. Therefore, *Beiji Qianjin Yaofang* contains plenty of ointment massage prescriptions, which can treat internal medicine, surgery, gynecology, pediatrics, ophthalmology and otorhinolaryngology, dermatology, and other diseases [11-12].

3.2.2 Wai Tai Mi Yao Fang (Essential Secrets from Outside the Metropolis) and ointment

Wai Tai Mi Yao Fang records many names of ointment massage prescriptions used to treat swelling,

arthralgia and pain. Most increased ointment prescriptions are used to treat surgical skin diseases, such as rheumatic pruritus, dandruff, alopecia, ringworm itching, and other diseases. *Wai Tai Mi Yao Fang* also emphasizes the technique of "Mozhiji" ointment after the application of massage ointment, described in *Wai Tai: Volume 32-Tou Feng Bai Xie Jian Shengfa Fang Bashou*. "Lianzicao juice, Songye, Qingtongbaipi, Zaogenvaipi, Fangfeng, Shaoyao, Baizhi, Xinhuangren, Zhenben, Chenxiang, Qinyuan, Shanglu root, Xijiaoxie, Qingzhupi, Xixin, Duruo, MANjingzi, Linglingxiang, Gansongxiang, Baizhum Tianxiong, Bobaipi, Fengxiang, Shengdihuang juice, Sesame oil, Zhuzongzi, Mazonggao, Xiongzhi, Manqingzi oil, The right 30 drugs are cut, mixed with Lianzi juice and Shengdi juice, soaked overnight. Then become the *Mozhizi* ointment." The purpose is to make drugs penetrate the subcutaneous muscle layer through the massage technique to improve the therapeutic effect of drugs. It provides an excellent reference value for future generations to study the development history of ointment massage therapy [13].

3.3 Song, Jin, and Yuan Dynasties

The ointment massage therapy in Song, Jin, and Yuan Dynasties was inherited from the Sui and Tang Dynasties, mainly through the local use of ointment massage therapy. In addition to using ointment massage to treat the diseased parts, it also cooperates with the theory of the Five internal organs, the Six hollow organs, and acupuncture points, and with massage techniques on the relevant parts and points. It can improve the clinical effect and has a remarkable curative effect.

3.3.1 *Tai Ping Sheng Hui Fang (The Peaceful Holy Benevolence Formulae) and ointment*

Tai Ping Sheng Hui Fang in the early Northern Song Dynasty was written in 992. Wang Huaiyin summarized the prescription of ointment massage and the experience of using ointment massage prescription. This literature includes the historical ointment prescriptions and the characteristics of special ointment massage applications. There are nearly 100 ointment formulas in the book, such as the ointment formula for treating eye diseases and the formula for treating eye diseases. Waist massage cream is very distinctive in its production and use. *Tai Ping Sheng Hui Fang* also records the "Mo Feng Gao" ointment, mainly used to treat Bi syndrome, and systematically summarizes the ointment massage therapy and external treatment of Bi syndrome such as gout. This book also records the "Mo Feng Gao" ointment, "Shen Yan Mo Feng Gao" ointment, and "Mo Tong Gao" that are widely used in orthopedics and traumatology departments. It is an excellent development of plaster

and moxibustion in treating orthopedic diseases. [14-15].

3.3.2 *Shengji Zonglu (The complete record of hoky benevolence) and ointment*

The record of Shengji was written from 1111 to 1117. The development of ointment massage therapy and application. The content of *Shengji Zonglu* is more complete than *Tai Ping Shengyi Fang (The Peaceful Holy Benevolence Formulae)*, which can be said to be a complete medical book in the Song Dynasty. This book has also made new contributions to the development of ointment. For example, "Mo Ting Gao" ointment treats eye diseases. *Tai Ping Shengyi Fang* is based on the basic theory of using an iron spoon as "Shengtie Tangdouzi" equipment. In *Shengji Zonglu: Volume 150-Chimaichongguanheijing*, use "Shengtie Tangdouzi" equipment with "Modingmingmugaofang" to treat the pinkeye that causes by the wind-heat pathogen. Moreover, *Shengji Zonglu* records many effective ointment prescriptions used in the treatment of orthopedics and traumatology and further integrates them into one of the three main orthopedics and traumatology treatment processes. It expands the clinical application of ointment massage in orthopedics and traumatology [16].

3.4 Ming and Qing Dynasties

In the Ming and Qing Dynasties, adult massage therapy was greatly limited due to the shackles of feudal ethics since the Song Dynasty. At the same time, pediatric massage has been comprehensively developed.

3.4.1 *Ben Cao Gang Mu (Compendium of Materia Medica) and ointment*

Li Shizhen has unique views on treating Apoplectic Hemiplegia with ointment massage therapy. The thorough analysis of cleavage, method, prescription, and medicine is his experience summary of ointment massage therapy. The clinical efficacy is outstanding and needs further research. Ben Cao Gang Mu records many ointment massage and instrument massage therapy in treating internal, surgery, gynecology, pediatrics, facial features, and dermatology diseases. The drugs recorded in Ben Cao Gang Mu include Yiyu, Huma, Huma oil, Wushi, Xiongzhi, Dongbitu, Longnaoxiang, Qicao, Spider, wine, Chi, Lichang, Jincai, Salt, and Maxie, etc. This book records the treatment of various diseases with ointment massage, such as dead fetus in the uterus, retained afterbirth, postpartum uterine prolapse, hypogalactia, galactostasis, childhood eclampsia, fever, infantile rhinorrhea, infantile craniectomy, jaundice, swelling, abscess, gall, Scrofula, Centipede and scorpion sting, fracture, abdominal pain, hemiplegia, headache, head wind pain, chest and rib pain, waist and kidney deficiency cold, urine obstruction, Prickly heat, The eyes look up and cannot rotate, cataract, choking, hair loss, beriberi, etc. Therefore, Ben Cao Gang Mu

summarizes Li Shizhen's personal experience and also notes all the ointment formulas before the Ming Dynasty [17].

3.4.2 Puji Fang (*Prescriptions for Universal Relief*) and ointment

In the Puji Fang written by Zhu Di in the Ming Dynasty, the ointment prescriptions recorded, such as "Wuwu Gancuo Shengmo ointment," "Taifu white ointment," and "Yannian Shuo Zhai ointment," can be seen in the historical documents before this book, which can be said to be another collation and summary of the classic ointment with good curative effect. For example, "Mo Yao pill" in Puji Fang: Volume 155 Shentimen-Wuzhongyaotonglun treats kidney yang deficiency and other five kinds of low back pain [18].

3.4.3 Li Yue Pianwen (*Topical Remedies in Rhyme*) and ointment

Wu Shangxian, a surgeon in the Qing Dynasty, wrote the Li Yue Pianwen, which has been pushed to a new peak in theory and clinical practice as a significant contribution. Wu Shangxian believed that "external treatment can treat internal diseases, internal medicine can be used as external medicine," and "ointment can cure all diseases." For internal governance, all kinds of decoction, pills, and powder are used as an ointment. For example, "Rubbing the waist for the disease of lower Jiao Method" and "Huo Mo Yu Dian" ointment. He believed that the ointment rub could be reduced to the needle burnt. From the guidance, exhalation, acupuncture, moxibustion, and ointment massage methods from Jin Gui Yao Lue (Synopsis of the Golden Chamber) proposed that "the pathogenic factor of apoplexy lies in the meridians and does not enter the viscera." He used "Da Bai" ointment and "Dahei" ointment from Qian Jin Yao Fang (*Prescriptions Worth a Thousand Pieces of Gold for Emergencies*) to treat leprosy. According to the theory that "external treatment can treat internal diseases, and internal medicine can be used for external medicine," Wu Shangxian used "Xiaoerjijingfengdingzi" ointment to treat infantile convulsion and "Xiaoqinglongjiashigao Decoction" to treat cough and asthma.

Conclusion

Different researchers often have different conclusions about the development of ancient ointment massage in traditional Chinese Medicine. Due to different priorities, differences are inevitable. This paper makes comprehensive-textual research on the relevant materials of ointment massage carried in the historical documents as far as possible and strives to comprehensively and objectively reflect the true face of history. However, ancient Chinese medicine was rooted in traditional Chinese medicine. Its development process was not synchronized with the overall

development of traditional Chinese medicine, which reflected the difference molding of different technical categories of the same discipline by the overall factors of culture, politics, and society. The cognition of this molding difference may be a good source for researchers to make different conclusions.

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Research progress in the treatment of Lumbar Disc Herniation with special acupuncture therapy

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ABSTRACT

Lumbar disc herniation (LDH) refers to a clinical syndrome in which the nerve root and cauda equina nerve are stimulated or compressed by the protruding intervertebral disc tissue on the pathological basis of LDH. Its clinical manifestations are low back pain, lower limb radiation pain, lower limb numbness and weakness, stool and urination dysfunction, etc. Most patients with lumbar disc herniation can improve their symptoms after conservative treatment. For those patients without significant nerve damage, the preferred treatment should be non-surgical treatment. There are many records about low back pain in traditional Chinese medicine literature, which are scattered in the discussion of "low back pain", "waist and foot pain", "waist and leg pain", "arthralgia syndrome" and other diseases and syndromes. Moreover, there are many kinds of therapies, especially the use of special acupuncture and moxibustion therapy for LDH has a good curative effect. Among them, all kinds of therapies have their own characteristics and continue to develop to the present. This paper introduces some literature reviews on the application of special acupuncture therapy in the treatment of LDH for reference, and the contents are summarized as follows.

Keywords: Lumbar disc herniation; Acupuncture; Special acupuncture; Research progress

Introduction

腰椎间盘突出症 (Lumbar disc herniation, LDH) 是指在腰椎间盘突出病理基础上, 由突出的椎间盘组织刺激或压迫神经根、马尾神经后所表现为腰痛、下肢放射性痛、下肢麻木无力、大小便功能障碍等的临床综合征。大部分腰椎间盘突出症患者经保守治疗后症状均能得到改善, 非手术治疗应作为不伴有显著神经损害的此类患者的首选治疗方法[1]。中医学中有关腰痛的记载很多, 散见于“腰痛”、“腰脚痛”、“腰腿痛”、“痹证”等病证的论述中[2]。其疗法有多种, 尤其是特种针法能对LDH取得了良好的疗效, 各有自己的特色, 且不断的发展到现在。现介绍一些特种针法治疗LDH的文献综述, 以供参考, 总结如下。

特种针法治疗

1. 浮针疗法

浮针疗法是符仲华老师在腕踝针及皮部理论等, 传统医学理论的基础上发明。该疗法以患肌为针刺靶点。是传统中医学和现代医学相结合, 使用针具在患肌周围进行皮下, 扫散, 辅以再灌注活动, 达到治疗目的。浮针虽来源于中医, 却是一种发展于基础医学的现代针刺方法, 浮针疗法的现代医学机制研究主要以 MTrP 为理论基础[3]。黄育聪等[4] 将80例LDH患者随机分为对照组和治疗组, 各40例, 对照组采用常规针灸治疗, 治疗组采用浮针疗法。治疗组(浮针组)应用一次性浮针, 治疗时先找肌化条索处及MTrP以定位, 进行浮针治疗。结果: 治疗10d后, 对照组总有效率为70.0%, 治疗组

(浮针组)为 97.5%，浮针组疗效明显高于对照组。随着浮针理论不断发展，一块患肌可以存在1个或多个MTrP，与LDH发病相关的“患肌”有很多，主要包括竖脊肌、腰方肌、腹外斜肌、多裂肌、臀肌、阔筋膜张肌、股二头肌、腓肠肌、腓骨长肌等[5]。朱佳会[6]采用浮针治疗LDH，浮针组选取患者腰背部、腹部及下肢患肌作为针刺靶点进行浮针治疗，结果：治疗2个疗程后，浮针治疗组VAS降低、JOA评分增高比较传统针刺更加显著，说明浮针疗法能够快速治痛、改善功能，具有显著的即刻效应。

2. 腹针疗法

腹针疗法是薄智云教授经过20多年的临床经验发明。是一种基于中医学基础理论，以神阙调控系统为核心，结合全息理论形成的一种针刺新疗法。腹针疗法治疗LDH根据薄智云经验，主穴为水分、气海、关元；配穴以随症加减：急性期加水沟、印堂穴；缓解期加气穴（双侧）；腰痛为主加外陵（双侧）、四满（双侧）；合并下肢痛加气旁（健侧）、外陵（患侧）、下风湿点（患侧）。张嫣然[7]将72例LDH继发坐骨神经痛患者，按照随机法分为治疗组(腹针组)和对照组，结合现代西医物理检测仪器，可观察两组的疗效，对照组针刺患处，取穴：大肠腧、腰夹脊、环跳、委中、阳陵泉、悬钟、丘墟等穴位。治疗组（腹针组）取穴：水分、气海、关元、气穴（双）、气旁（健侧）、外陵（患侧）、下风湿点（患侧）、下风湿下点（患侧）。两个疗程后治疗组（腹针组）的肌电图（对比治疗前肌电图当中的胫神经与腓总神经运动神经传导速度及波幅），治疗指数效果均高于对照组。林安、梁超[8]采用腹针治疗LDH。方法：对比两组的LDH患者治疗后临床疗效，结果：腹针组的中医症候积分、VAS评分、Oswestry评分和JOA评分指数明显优于对照组。腹针组的IL-6、CRP和TNF- α 水平显著低于对照组。

3. 铍针疗法

铍针是古代九种针具之一。《九针十二原》记载：“铍针者，末如剑锋，以取大脓”，铍针的形态是一个类似于剑的针具，铍针的末端有刃，主要作用为“取大脓”。铍针是由董福慧教授的临床经验，结合中医微创理念，把传统治疗工具“铍针”进行了改良。治疗机制能对肌肉、筋膜和皮下组织的进行局限分离，减轻筋膜表面张力，降低筋膜腔压力，加快患区局部新陈代谢速度，同时也能给局部毛细血管扩张，无菌性炎性物质可更快吸收，从而缓解疼痛[9]。杨康等[10]认为LDH此病发作时，腰腿疼痛明显，多有血瘀痹阻，以采用铍针疗法结合口

服身痛逐瘀汤，能改善围血液循环，消除局部水肿疼痛缓解较快，不良反应小。达到扶正固本、化瘀通络、通则不痛的目的，进一步治疗方案的进展。

4. 平衡针疗法

平衡针疗法是指在中医学的基础上，结合现代科学理论而形成的一种新型针法。其理论主要源于中医阴阳学说、经络学说、心神调控学说、中枢神经调控原理及穴位生物全息律。以通过刺激穴位及神经支，将信号传输到大脑中枢调控中心，从而迅速调节中枢递质，对病灶部位进行调控，以达到镇痛效果[11]。李霞等[12]将60例LDH患者随机分为方法平衡针组和传统针组。观察平衡针治疗LDH，平衡针组选取腰痛穴（前额正中直上1.5寸）按照患者疼痛部位，进行平刺进针，针感以酸、胀、沉时，就可出针。结果：平衡针组VAS指数和疗效均优于传统针组。伍素科，张平[13]采用平衡针治疗LDH的腰腿痛。方法：观察组应用平衡针法，选用腰痛穴，进针平刺3cm针方向根据患者具体部位，若腰部左侧疼痛则针尖向右刺，右侧疼痛则针尖向左刺，腰脊正中疼痛则针尖向下，手法进行提插捻转，针感以酸麻胀为主。结果：经过1个疗程治疗后，观察组患者治疗总有效率为93.6%，明显高于对照组的84.0%。平衡针疗法治疗LDH的效果明显，安全性好，在针灸治疗LDH方面，常规针灸可结合平衡针疗法以提高疗效，改善患者的临床症状。

5. 腕踝针疗法

腕踝针疗法是上海中医学院张心曙教授于1975年创用。是以经络理论与神经反射理论相结合，把身体两侧分6个纵区，在腕及踝部各定6个进针点，用数字1到6进行编号。根据病症的表现，寻找压痛点在对应区域的腕踝部进行针刺治疗。对于LDH患者，治疗选择腕踝针以找准压痛点为关键。此类患者大都在腰夹脊穴附近及坐骨点处有压痛点，因此选择压痛点的同侧下4，下5，下6作为针刺点。陈静等[14]研究腕踝针对LDH术后的镇痛疗效及功能康复的影响。将60例LDH术后患者分为观察组和对照组。对照组常规护理和消炎镇痛药，观察组在对照组基础上应用腕踝针治疗。治疗后2周对VAS、JOA和ODI两组的指数均为良好，尤其是治疗组的临床总有效率85.7%明显优于对照组。说明腕踝针疗法可提高LDH镇痛效果。陈琦、邵彬彬[15]探讨电针腰夹脊穴结合腕踝针治疗LDH的临床价值。将患者80例，随机分成两组。治疗组采用电针腰夹脊穴结合腕踝针，对照组采用常规治疗。治疗组总有效率95.00%显著高于对照组。

6. 头针疗法

头针疗法（国际头皮针），是在头部特定的穴位线，进行刺针的一种方法。头针的理论依据主要有二，一是根据中医传统的脏腑经络理论，二是根据西医大脑皮的功能。定位在头皮的反射区联

合传统针灸的头部穴位线，相应取穴。虽然头针是一个独立的系统被提出仅有几十年的时间，但头穴治疗疾病却有几千年的历史。最早在《内经》中就有指出：“头为诸阳之会”，手足三阳经循行经过头，**阴经**则通过经别间接地与头部相联系，《素问·脉要精微论篇》提出：“头为精明之府”，凡五脏六腑的精气均上注于头[16]。陈迎春,冯祯根[17]观察头针结合电针夹脊穴治疗LDH的临床疗效。方法：选取LDH患者86例,随机分为观察组和对照组,对照组以电针夹脊穴治疗,观察组以头针结合电针夹脊穴治疗。观察组在对照组基础上加头针治疗，采用国际头针,取头部顶中线、顶旁1线、枕上正中。按常规沿皮针至帽状腱膜下,留针30分钟。结果：治疗后观察组优良于对照组效率为88.4%。

7. 其他特殊疗法

7.1 刺络拔罐

刺络拔罐是通过放出富含疼痛物质的血液，改善局部微循环有利于修复受损组织，且加上火罐的负压及温热作用。可促进血管扩张以改善微循环，使得LDH的腰部患肌血液微循环加快，加强舒经通络祛瘀新生之力，从而能减轻LDH的症状[18]。杜春蕾,林杨[19]将LDH 90例患者随机分成治疗组和对照组，对照组采用传统针灸结合口服身痛逐瘀汤加**减**45例，治疗组是以对照组基础上加刺络拔罐45例。结果：两周之后发现腰痛减轻有效率分别为：对照组88.89%、治疗组97.78%，直腿抬高试验阴性有效率分别为：对照组84.44%、治疗组95.55%。两组均有良好的疗效，尤其是治疗组，可说明刺络拔罐能提高临床疗效。

7.2 针刀疗法

针刀是通过发挥刀刀剥离的作用，松解黏连的神经，解除脊神经根的压迫，达到活血镇痛及消炎的作用。最主要是小针刀操作者，必须对人体解剖，肌肉骨骼，血管神经的分布走向要有掌握和熟悉程度。因此该治疗法的操作者需具备孔实的基本功，才能尽可能的避免不良反应的发生。施颖初等[20]采用针刺联合小针刀治疗LDH。观察组以小针刀结合针刺LDH相关穴位。结果：治疗1周、4周后组JOA评分逐渐升高，ODI评分逐渐降低两组都取得良好效果。尤其是针刀联合针刺组的疗效高于对照组。说明针刺联合小针刀治疗LDH效果显著，能降低炎症因子水平，缓解疼痛症状，改善患者腰椎功能。

7.3 穴位注射

穴位注射疗法是指在针刺疗法基础上给予药物治疗。施颖初等[21]研究表明，穴位注射将针刺跟药物作用有机结合，能够降低IL-6、NO水平，促进炎症因子的吸收，有利于改善LDH临床症状，是临床上的常用治疗手段。李江等[22]对120例LDH进行临床研究，均选择大杼、委中**两**穴，对照组用普通针刺，治疗组采用穴位注射，治疗后结果提示

治疗组症状改善程度明显优于对照组。

7.4 温针疗法

温针灸始见于《伤寒论》，又称为温针、柄柄灸和烧针柄等，是一种将普通针刺与艾灸相结合的治疗方法。艾灸的温热效应可通过针体传到关节深处，不仅起到温阳散寒通络之效，又可起到活血化瘀止痛之功。朱斌 [23]采用温针疗法治疗LDH患者。随机分为传统温组法和普通针刺组。经过治疗一个月后，**两组**患者VAS评分、JOA评分、ODI评分比较，相比治疗前都显著改善，即**两组**治疗均为有效治疗，且传统温组法随着时间变化改善情况优于普通针刺组。

Conclusion

以上文献表明，特种针法治疗LDH有多种，各有特色，疗效均取良好效果，从文献报道看，大部分都有效率80%以上。无论是浮针疗法、腹针疗法、头针疗法、铍针疗法、平衡针疗法、腕踝针疗法等或是其它治疗方法，均取了良好效果。另外也存在一些问题从收集的文献资料来看，临床研究及其机理机制等研究及疗效、疗程标准缺乏统一性，且医家的施针手法、患者病情等这些因素是否有影响，还有待研究，尽早解决病人的痛苦。

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Case Report: Treating Atopic Dermatitis with Si-Wu-Xiao-Feng Decoction in combination with Topical Huang Qin Cream

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ABSTRACT

The aim of this study is to evaluate the efficacy of treating Atopic Dermatitis with the Traditional Chinese Medicine (TCM) Formula of Si-Wu-Xiao-Feng decoction in combination with Topical Huang Qin Cream. The case report is from a 42 years old female patient presented with lesions of small blisters bilaterally spread all over her arms and legs. The blisters often become very itchy and has affected the patient for over 5 years. The blisters became more severe during the past 2 months. She is prescribed Si-Wu-Xiao-Feng decoction to drink daily 30 minutes after breakfast and dinner. The TCM herb composition of the Si-Wu-Xiao-Feng Decoction is adjusted after each follow up according to the severity and condition of the blisters. She is also prescribed a Topical Huang Qin Cream. This is an extemporaneous formulation prepared by the Institute of Thai-Chinese Medicine Mae Fah Luang University. After 6 months of treatment, the lesions of blisters, papules and scaly skin has recovered back to normal skin. After treatment was discontinued, the patient was followed up at 2 weeks and shown no eruption of blisters or signs of itchiness. Moreover, the patient liver and kidney function were measured at the 3rd month during the treatment. All parameters were within normal range. In conclusion, treating Atopic Dermatitis with the combination of Si-Wu-Xiao-Feng decoction and Topical Huang Qin Cream has shown good efficacy. It also shown potential in preventing the recurrence of the disease and is safe for the patient. This treatment regimen should be studied in a wider clinical setting for developing new treatments for Atopic Dermatitis patients in the future.

Keywords: *Atopic Dermatitis; Chinese Medicine; Huang Qin; Si Wu Xiao Feng Decoction*

Introduction

Atopic Dermatitis is a chronic skin disease which is commonly found in children. The main clinical features are itchy, dry scaly skin which recurs periodically [1]. It is considered a common non-communicable disease. It is reported to occur worldwide with a prevalence of 20% in children and 2-8% in adults. Symptoms often develop in children. In severe cases the symptoms persist into adulthood [2,3]. It can be categorized into 2 type which are intrinsic type or the non-igE associated and the extrinsic type of the igE associated. Patient of the latter type are shown to have

high serum igE levels and have a positive skin prick test [4]. According to TCM, Atopic Dermatitis is known as Shi Chuang (湿疮). There are many etiologies of Shi Chuang such as a congenital physical imbalance and inappropriate eating routine. Consuming certain types of food such as spicy food and meat can increase the heat in the body which affects the function of the stomach and spleen. This can cause imbalance in digestion, nutrient absorption and nutrient dissemination which increases the heat and dampness in the body. Moreover, the body is also exposed to wind which is an external factor. When the wind, heat and dampness (external and

internal factors) spreads into the skin the disease will exacerbate [5]. According to TCM, Atopic Dermatitis can be categorized into 3 types of syndrome [6] are as followed:

1. dampness-heat syndrome clinical feature: acute lesion: erythema, swelling, wheal, itchiness most of the time, blisters with fluid leakage upon rupture concurrent symptoms: tachycardia, thirsty, hard stool, yellow urine, redness color of tongue, yellow and sticky plaque on tongue, rolling pulse

2. spleen deficiency and dampness retention syndrome

clinical feature: slow progression of the disease
lesion: mostly are papules and vesicles with darken and pale spots, scaly skin, itchiness, minimal fluid leakage upon rupture from scratching
concurrent symptoms: loss of appetite, loose stool, polyuria with clear or light-yellow color, pale tongue, white or sticky plaque on tongue, soggy pulse

3. Blood-deficiency and wind-dry syndrome
clinical feature: long progression of the disease with on and off episodes

lesion: lichenification, severe itchiness, scaly skin, small erythema rashes

concurrent symptoms: pale tongue, thin plaque on tongue, thready pulse

The treatment regimen of the formula and herb ingredients are adjusted according to the syndrome and concurrent symptoms of the patient at each visit.

Si Wu Xiao feng Decoction [7] is a TCM formula for treating chronic Atopic Dermatitis or the common syndromes of Blood-deficiency and wind-dry syndrome. It has shown to have good efficacy. The main properties are nourishing blood, increase dampness, remove wind and reduce itchiness. The ingredients are as followed:

1. Monarch drug (jun yao): *Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey. – reduce heat, cools the blood and nourish yin; *Angelica sinensis* (Oliv.) Diels and *Ligusticum chuanxiong hort* – nourishing blood, improve circulation and harmonizes yingfen and weifen.

2. Ministerial drug (chen yao): *Saposhnikovia divaricate* (Turcz.) Schischk, *Nepeta cataria* L. and *Heracleum hemsleyanum* Diels in combination – remove wind and reduces dampness for reducing external factors and reduces itchiness; *Cryptotympana pustulata* Fabricius and *Mentha haplocalyx* Briq. – remove wind, provokes rash and reduces itchiness.

3. Adjuvant drug (zuo yao): *Polygonum multiflorum* Thunb. - nourishing liver and kidney, tonifying and replenishing blood and essence, enrich yin; *Cannabis sativa* L. - moistening dryness and simulate the circulation of blood. These 2 herbs can potentiate the monarch drug in nourishing yin and blood; *Dictamnus dasycarpus* Turcz. – reduces heat and dampness, eliminates the dampness in the skin; *Paeonia*

lactiflora Pall. – nourishing blood and harmonizes yingfen and weifen

Huang Qin Cream is an extemporaneous topical cream for treating skin conditions which is prepared and used exclusively by the Institute of Thai-Chinese Medicine Mae Fah Luang University Hospital. The main indication is for skin rash with inflammation and itchiness which are a non-infectious cause. The main herb ingredient of *Scutellaria baicalensis* Georgi. is shown to have many biological properties such as fatigue reduction, antibacterial, anti-inflammatory and immunomodulation [8]. Other ingredients of Vaseline, glycerin and lanolin have moisturizing effects.

Case Report

1st visit: 42 years old female patient came to the General Medicine Department of the Institute of Thai-Chinese Medicine Mae Fah Luang University Hospital. Patient has a history of Atopic Dermatitis with on and off episodes since 2016. Patient had received treatment from a dermatological clinic with a type of injection, oral antihistamine and topical medications. The skin rash and itchiness were relieved after the treatment but exacerbated after exposure to external stimulants. In this visit, patient has lesions of small blisters bilaterally spread all over her arms and legs. The itchiness is very severe in the ankle area. She has had these symptoms for over 3 months and it has become severe during the past month. Patient reported to have a underlying disease of allergic rhinitis. Patient reports no severe disease, history of surgery, drug allergy, consumption of alcoholic beverages, smoking and family history of any severe diseases.

Physical examination of the rash on both arm and legs showed erythema rash with some darken spots. Prolonged rash mostly became dry, scaly, swelling and thick. Some of the newly erupted rashes are blisters with minimal fluid leakage. Most rashes are itchy but within tolerable limits. Itchiness is increased after consumption of spicy food, exposed to hot weather and increased sweating. Patient often feels thirsty and prone to cold sores but has low consumption of water. Normal diet and normal bowel movement. Difficulty sleeping and increased itchiness during her sleep cycle. She often feels fatigue after waking up. Tongue has reddish color with a thick white plaque. LMP 26.6.2564*6 days with normal amount, reddish color, minimal blood clots and no menstrual pain.

Diagnosis:

Conventional medicine - Atopic Dermatitis (Chronic Stage)

TCM - Shi Chuang (Blood-deficiency and wind-dry syndrome)

Treatment: aim to nourish the blood, increase dampness, expel wind and reduce the itchiness. Oral medicine is the modified Si-Wu-Xiao-Feng decoction and topical medicine is the Topical Huang Qin Cream.

Modified Xiao-Feng decoction should be taken 30 minutes after meal. Take 180 ml per dose after breakfast and dinner. Duration 7 days.

Topical Huang Qin Cream should be used after bath in the morning and evening or when itchiness increases. Duration 7 days.

Follow up is after 7 days of the current visit and advised to avoid spicy food, oily food, salty food and fried food. Get enough rest and exercise regularly.

2nd visit: After using the oral and topical medicine for 1 week, patient reported no side effects from the medication. Newly erupted blisters on the arm and leg are still present with tolerable level of itchiness. Itchiness increases with hot weather or when excess sweating. Diet and bowel movement is normal. Falling asleep and sleeping duration improved. Dry mouth but reduces thirstiness. Tongue is pale red with thick white plaque. Small pulse.

Diagnosis reveal that the patient has newly erupted rash due to the increase heat according to TCM. Therefore, the herb ingredient *Paeonia suffruticosa* Andr. Was added into the modified Xiao-Feng decoction formula to help clear heat and cool the blood. Topical Huang Qin Cream was prescribed like before. Duration of the treatment regimen is 14 days and follow up was appointed after 2 weeks.

3rd Visit: After the treatment, patient showed to have no new eruption of the blister rash. The recovered rash are mostly dried out but felt to have good moisture. Itchiness is increased especially during night time. Sleeping pattern disturbed due to the itchiness. Diet and bowel movement is normal. Tongue is pale red with thick white plaque. Small pulse.

The herb ingredient *Tribulus terrestris* L. was added to help expel wind and reduce itchiness. While the herb ingredient *Nepeta cataria* L. was removed from the modified Xiao-Feng decoction. Topical Huang Qin Cream was prescribed like before. Duration of the treatment regimen is 14 days and follow up was appointed after 2 weeks.

Now the patient is at the 1 month of the treatment. No new rash eruption was reported. Previous papule rashes are now thinner and most of the darken spot have become lighter. Itchiness has reduced with some areas of dry skin. Patient reported that the topical Huang Qin cream can completely reduce the itchiness. Other vital signs are normal. Afterwards, the modified Xiao-Feng decoction composition are adjusted according to the syndrome at each visit which the patient is appointed for follow up every 2 weeks.

After 3 months of the treatment, the patient's liver and kidney function was measured. Laboratory results showed that AST = 17 U/L, ALT 18 U/L, ALP 61 U/L, BUN 10.7 mg/dL and Creatinine 0.8 mg/dL. All parameters were within normal limits therefore the treatment with TCM medications was allowed to continue.

After 6 months of the treatment with modified Xiao-Feng Decoction and Topical Huang Qin Cream, patient's symptoms have gradually recovered. No new rash eruption. Previous darken papules have become thinner and lighter. No itchiness. Therefore, the treatment was discontinued. Discharge advices were to avoid spicy food, oily food, salty food and fried food because they can increase heat in the body. Avoid any known allergy stimulant. Get enough rest and exercise regularly.

Telemedical followed up after the discontinuation of the treatment at 2 and 4 weeks. Patient reported no new rash eruption and no disturbance in her daily life.

Discussion

According to TCM, Atopic Dermatitis (Chronic Stage) can be categorized into spleen deficiency and dampness retention syndrome and Blood-deficiency and wind-dry syndrome [9]. The patient in this case has a long progression of the disease. Lesions are erythema and papule rash with some darken spots. Severe itchiness. Area of the elevated skin in the rash are harden and scaly. Tongue is pale and pulse is small. These symptoms correlate with the condition of Shi Chuang with the blood-deficiency and wind-dry syndrome. The etiology of this condition is caused by the chronic nature of the disease. Heat-dampness stagnation inside the body, dampness stagnation which causes blood stagnation which is combined with the heat inside the body [10]. This contributes to the intense stagnation of heat, dampness and blood stagnation in the later stage. The wind and heat destroys the Yin. This causes the dampness stagnation that obstructs the Luo meridian and reduce the blood circulation to the skin. These all contribute to the blood-deficiency and wind-dry syndrome. According to TCM, the treatment for Shi Chuang with the blood-deficiency and wind-dry syndrome must enrich blood, moisten dryness, expel wind and stop itchiness. In this case the researcher uses the TCM formula of Si-Wu-Xiao-Feng decoction as the main medication. In case of severe itchiness which disturbs the sleeping pattern, the herb ingredient *Ostrea gigas* Thunberg and *Hyriopsis cumingii* (Lea) was added. If there is blood stagnation, the herb ingredient *Salvia miltiorrhiza* Bge and *Corydalis yanhusuo* W. T. Wang was added. If the dampness is high, the herb ingredient *Dioscorea tokoro* Makino is added. If wind is high, *Tribulus terrestris* L. is added [11]. Oral treatment is not only a part of treatment for this disease. There are other treatments such as herbal paste, herbal shower or soaking, moxibustion, fire-needle therapy, acupuncture, cowherb attachment on earlobe, injection according to acupuncture point and blood pricking and cupping [12]. Behavioral adjustment is also an important part of the patient treatment plan. TCM doctors should advise patients to consume food with minimal seasonings. Avoid spicy food, fried food and other food that can

cause cold sore. Avoid foods that are known to cause allergy such as seafood and fermented food. Avoid tea and coffee.

Conclusion

This case report has demonstrated the effectiveness of the diagnosis and treatment of Atopic Dermatitis (Chronic Stage) according to TCM. TCM doctor must be able to provide a correct differential diagnosis of the condition or syndrome of the disease. The next important point is the selection of an appropriate TCM formula must be selected with adjustment of the herb ingredient amount and composition according to the dynamic of the disease condition throughout the treatment plan. Improper adjustment, such as non-compatible formula or using the herb ingredient in subtherapeutic or suprathreshold levels, can greatly affect the efficacy of the treatment or even cause undesired adverse effects. This would also be a burden on the patient in terms of medical cost. Treating Atopic Dermatitis (Chronic Stage) with TCM requires a long period of time and the patient must have good discipline and patience during the treatment plan. The results of the treatment is a reduction in the exacerbation of the disease, relief from the chronic skin lesions and these treatment also have minimal side effects on the major metabolic organs. However, this is a single patient study. To improve this treatment regimen, a clinical study in a larger population is required. Moreover, pharmacological studies on the efficacy of Si-Wu-Xiao-Feng decoction for treating Atopic Dermatitis (Chronic Stage) should be investigated. These data are essential for developing new clinical standards for the treatment of Atopic Dermatitis (Chronic Stage) with TCM in Thailand in the future.

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The Effectiveness of Thai Traditional Massage in Improving Constipation at Institute of Thai-Chinese Traditional Medicine Hospital, Mae Fah Luang University

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ABSTRACT

Introduction: At present, constipation is most common in the world, including in Thailand, and if left for a long time. This can lead to many complications and affect daily life. Today, the treatment for constipation most of them uses laxatives. Long-term use of laxatives can cause drug resistance. Therefore, the purpose of this study is to study the effectiveness of Thai traditional massage in improving constipation and the study satisfaction with the treatment of chronic constipation.

Methodology: This study was a one group study design, clinical trial in patients with chronic constipation. The research participants were female or male, aged 20 - 45 years, residing in Chiang Rai province. Thirty subjects had constipation according to Rome III criteria and were diagnosed with constipation that wasn't caused by mechanical obstruction or due to another cause such as cancer by a modern physician. All will be treated with Thai traditional massage and herbal compresses to treat chronic constipation for a period of 1 day and collected data with measuring instruments, including a recorded form.

Results: The study showed improved behavior of defecation, urination, and decreased use of laxatives. There was an increase in the number of times defecation. Difficulty in defecation, inability to defecate, abdominal pain during defecation, time to use defecate, and use of laxatives or manual maneuvers to facilitate defecations are decreased. In addition, the satisfaction of the research participants in terms of quality of service, quality of treatment, and overall satisfaction level were strongly satisfied.

Conclusion: The study had shown that Thai traditional massage for chronic constipation helps to stimulate the frequency of bowel movements without any side effects. It was concluded that the Thai traditional massage was safe and suitable to be used to treat primary constipation.

Keywords: *constipation, chronic constipation, Thai traditional massage*

Introduction

At present, constipation is most common in the world, including in Thailand. The most common group is between the ages of 20 and 40 [1]. Chronic constipation most patients will have symptoms of having lumpy or hard stools, rarely feeling pain to

defecate, taking a very long time to the stool, or failing to the stool [2]. In addition, environmental factors may cause constipation, such as housing, cold weather, or the economy.

There are both medicinal and non-medicated methods of treating constipation. Most drug-based

treatments are laxatives, but should not be used continuously for the long term, which can cause drug resistance [3], and non-medicine treatment such as behavior modification drinking plenty of water, at least 1.5 to 2.0 liters per day, light to moderate daily exercise may increase bowel movements. [2]

In Thai traditional medicine Constipation is likened to Dan-Lom. Incomplete defecation, difficulty in defecation, feces hard like goat feces. [4]. Herbal compress relieves pain, reduces muscle spasms, improves blood circulation. and the aroma of herbs helps to relax.

Chronic constipation if left untreated for a long time. It can lead to systemic diseases, such as colorectal ulcers, hemorrhoids, etc. Including affecting the quality of life's daily life [5]. The researcher is interested in studying the effectiveness of Thai traditional massage in improving constipation because in the treatment of chronic constipation there are many studies, including both medicinal and non-medicated methods of treating constipation, medicinal [6] herbs [7], and self-acupressure on pregnancy-related constipation [8]. However, the effectiveness of Thai traditional massage in improving constipation in Chiang Rai province has not been studied, and if Thai Traditional massage can be used to affect the treatment of patients with chronic constipation. It can reduce the complications of chronic constipation and improve the quality of life. Therefore, the purpose of this study is to study the effectiveness of Thai traditional massage in improving constipation. The purpose of this study is to study the satisfaction with the treatment of chronic constipation and explore the treatment options for chronic constipation.

Methodology

Study design

This study is a one group study design, clinical trial in patients with chronic constipation.

Population and sample size

30 volunteers with Rome III chronic constipation.

Data collection

The researcher developed the assessment to study the Thai traditional massage efficacy for chronic constipation at Institute of Thai-Chinese Traditional Medicine, Mae Fah Luang University. The instrument used to measure results is a data record and data were compared before and after the experiment and then imported into the statistical analysis program IBM SPSS STATISTIC 21.

Operating area

Institute of Thai-Chinese Traditional Medicine 2nd floor, Department of Orthopedic and Sports Medicine

Mae Fah Luang University, Chiang Rai and there were 30 research participants.

Population size

Thirty volunteers with publicized constipation (Dan-lom) were interested in participating in the study.

Inclusion criteria

1. People aged 20 - 45 years.
2. Male or female.
3. People with constipation by Rome III criteria have 2 or more symptoms for more than 3 months.
4. History taking and physical examination to diagnose constipation that is not caused by mechanical obstruction or from other causes such as cancer (examined by a modern physician).

Exclusion criteria

1. Pregnant or breastfeeding women.
2. Patients with Hemiplegia or Paresis.
3. Contagious skin diseases such as chickenpox, shingles, contagious leprosy, etc.
4. Volunteers cannot communicate, understand or follow instructions.
5. Fever higher than 38.5 °C or menstruation on the day of treatment participation.
6. Uncontrolled high blood pressure (Uncontrolled hypertension) blood pressure is greater than 170/90 mmHg.
7. There is thrombosis. and clots with blood clots (Thrombophlebitis).
8. Taking anticoagulant drugs such as Aspirin, Clopidogrel, Prasugrel, Ticlopidine, etc.
9. Patients who treat constipation with medication.
10. Haven't taken antibiotics in the past 3 months.

Discontinuation criteria

1. Cardiovascular system abnormalities, such as chest pain, etc., or urinary system abnormalities such as Dysuria and Hematuria, etc.
2. A medical condition that develops during research is found to be life-threatening if it is still in the research process and is not treated.
3. Failure to comply with research rules or fail to follow up as scheduled.
4. The volunteers want to withdraw from the research project.
5. How to reach volunteers, such as inviting doctors or nurses, or placing advertisements.

Research tool

The instruments used in this research consist of 2 parts: research tools and tools used to collect data.

Methodology

The experimental group consisted of 30 people treated according to the traditional Thai medical practice guidelines, consisting of Thai traditional massage and herbal compress.

Treatment evaluation

The experimental group will be assessed using a record of excretion behavior, constipation before and after treatment, adverse reactions and assessed for their satisfaction with the treatment in terms of quality of service, quality of treatment, and overall satisfaction. After completing the study.

Outcome measurement

A questionnaire to assess the excretion behavior of the volunteers to assess the severity of the constipation of the volunteers.

Statistical methods for data analysis

1. Analyze general data using descriptive statistics, including mean and standard deviation of volunteers.
2. Data used for comparative testing, Intention to treat analysis with one-way ANOVA repeated measurement.
3. Analyze the results of each indicator Comparison between before the start of the experiment and after the experiment using paired-samples t-test statistics.

Results

From data analysis, it was found that most of the research participants were women. The most aged between 21-25 years and single status. Occupation of most of the students. Have had chronic constipation for 1-3 years, most of them. Most of the family members had no history of constipation. Most of the meat was eaten. The food tastes were mostly sweet, followed by salty flavor, spicy, and sour taste. The frequency of eating fatty foods Most were 3–5 times/week. Stress, most of them had sometimes to stress. Sleep soundly most of them sleep soundly sometimes. Most of them liked to eat salty food. Drinking tea and coffee, most of them drank sometimes (Table 1).

From data analysis, it was found that after the experiment defecation and urination behavior are better. Reduce the use of laxatives (Table 2).

Table 1 shows the general information of the samples

Sample group	(n=30) %	\bar{X} (S.D.)
Gender		1.63(0.49)
Male	11(36.7)	
Female	19(63.3)	
Age (years)		4.9(0.30)
16-20 years	14(46.7)	
21-25 years	16(53.3)	
Status		1(0.00)
Single	30(100)	
Religion		6.86(0.83)
Buddhism	29(96.7)	
Christianity	1(3.3)	
Occupation		1.62(0.48)
Personal business/ trade	3(10.0)	
Student	27(90.0)	
Have had chronic constipation for a long time (years)		1.10(0.31)
1-3 years	25(83.3)	
4-6 years	2(6.7)	
7-9 years	3(10.0)	
Family history of constipation		6.86(0.83)
Yes	5(16.7)	
No	25(83.3)	
Eating behavior		2.0(0.00)
Meat	30(100)	
Food taste		1.53(0.86)
Sweet	19(63.3)	
Salty	8(26.7)	
Sour	1(3.3)	
Spicy	2(6.7)	
The frequency of eating fatty food		2.23(0.43)
3-5 times/week	23(76.7)	
Everyday	7(23.3)	
Stress		2.33(0.47)
Sometimes	20(66.7)	
Regularly	10(33.3)	
Sleep soundly		2.23(0.43)
Sleep soundly sometimes	23(76.7)	
Sleep soundly every night	7(23.3)	
like to eat salty food		1.90(0.40)
Yes	25(83.3)	
No	5(13.3)	
Drink tea and coffee		1.96(0.41)
No	3(10.0)	
Sometimes	25(83.3)	

Table 2 Shows a study on the effectiveness of Thai traditional massage in improving constipation in before and after the experiment.

Excretion behavior	Before experiment		After experiment	
	\bar{X}	S.D.	\bar{X}	S.D.
Defecation	2.30	0.70	1.70	0.79
Urination	1.43	0.72	1.80	0.76
Use of laxatives	1.56	0.50	1.66	0.47

Table 3 Shows the results of a comparative study on the effectiveness of Thai traditional massage in improving constipation before and after the experiment.

Defecation characteristics	Before experiment		After experiment		df	t	P-value
	\bar{X}	S.D.	\bar{X}	S.D.			
Number of times of defecation	1.60	0.81	1.43	0.85	58	0.77	0.443
Difficulty in defecation	3.36	1.12	2.70	0.91	58	2.51	0.015*
Inability to defecate	3.23	1.04	2.70	1.02	58	2.00	0.050
Abdominal pain during defecation	3.00	1.01	2.16	0.91	58	3.34	0.001*
The time to use defecate	1.80	0.92	1.76	1.00	58	0.13	0.894
Use of laxatives or manual maneuvers to facilitate defecations.	2.00	0.58	1.83	0.83	58	0.89	0.375

* Statistically significant at p-value < 0.05

From data analysis, it was found that the researcher's participants were able to excrete within 1 day of experiment (Table 4).

Table 4 Shows the results of a comparative study on the effectiveness of Thai traditional massage in improving constipation before and after the experiment.

Date of first defecation	N	Percent
Day 1	23	76.7
Day 2	6	20.0
Day 3	1	3.3

From data analysis, it was found that most of them did not experience any adverse reactions. (Table 5).

Table 5 Shows the adverse reactions after the trial of Thai traditional massage in improving constipation.

The adverse reactions	N	Percent
Nausea		
Yes	2	6.7
No	28	93.3
Dizziness		
Yes	4	13.3
No	26	86.7
Loss of appetite		
Yes	7	23.3
No	23	76.7
Stomachache		
Yes	6	20.0
No	24	80.0
Pain from massage		
Yes	7	23.3
No	23	76.7
Burn after herbal compress		
Yes	0	0

From data analysis, it was found that after the experiment the researcher's participants had better defecation characteristics, especially the difficulty in defecation and abdominal pain during defecation were statistically significant at p-value < 0.05 (Table 6).

Table 6 Shows the results of the satisfaction assessment.

Assessment	Thai traditional massage		Satisfaction level
	\bar{X}	S.D.	
Quality of service	4.55	0.59	Strongly satisfied
Quality of treatment	4.55	0.59	Strongly satisfied
Overall satisfaction	4.63	0.51	Strongly satisfied
Total	4.55	0.59	Strongly satisfied

Discussion

From a research study on the effectiveness of Thai traditional massage on the treatment of chronic constipation. It was found that the participants in the study had better stool excretion. Decreased use of laxatives There is an increase in the number of defecation times. Decreased difficulty in defecation. Decreased incontinence symptoms. Abdominal pain during defecation decreased. Less time spent driving and the use of drugs or procedures was reduced. Past studies have shown that massage can help loosen muscle contractions in the gastrointestinal tract, thereby improving the digestive system. And the functioning of the intestines is efficient including being able to drive fluently and more time [9]. Thai traditional massage has a good effect on stimulating the frequency of excretion. Thus, it was concluded that Thai traditional massage was effective in treating chronic constipation. and suitable for treatment [10] The researcher's participants were able to pass bowel movements within day 1 of

treatment 76.7%, followed by day 2, 20.0% and day 3, 3.3%. The most of researcher's participants did not experience any adverse reactions after treatment. Some researcher's participants had nausea, 6.7%, dizziness, 13.3%, loss of appetite, 23.3%, stomachache, 20.0%, pain from massage 23.3%. Gastrointestinal decrease similar to the results of a past research study [11].

Conclusion

The conclusion of the research indicated that the Thai traditional massage was effective in treating constipation. Observed from defecation behavior before and after treatment, but massage can be dangerous, if the therapist is not properly trained. Treatment should only be done by a registered and licensed practitioner such as a practitioner of Thai traditional and applied Thai traditional medicine.

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The Effect of Court-Type Thai Traditional Massage on Delayed-Onset Muscle Soreness in The Biceps Brachii Muscle After Resistance Exercise

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ABSTRACT

Introduction: Delayed onset muscle soreness (DOMS) when they perform a different type of sport or practice new skills. DOMS is different from the acute pain experienced during and shortly after exercise. It usually appears after a pain free period (12–24 h), peaks at 24–72 hours and disappears within 7 days of the exercise.

Objective: To investigate the Effect of Court-Type Thai Traditional Massage (CTTM) on DOMS in The Biceps Brachii Muscle After Resistance Exercise.

Methods: Mae Fah Luang University student female, aged 18-22 years, 30 people. This was a Randomized Control Trial study. The volunteers were divided into 2 groups, the first group was a Court type Thai massage experiment with 15 people and the second group was a control group of 15 people. The subjects tested the Visual Analogue Scale (VAS), Range of Motion (ROM) and swelling were measured before, immediately and 24, 48 and 72 hours after the exercise.

Results: The results showed by comparing the differences between the groups, it was found that there was a statistically significant decrease in pain level ($p < 0.05$) at 48 hours. Range of motion of the experimental group there was statistically increased ($p < 0.05$) at 72 hours. The swelling of the muscles was not statistically in both groups and between groups.

Conclusion: CTTM can help muscles relax, reduce muscle contraction and reduce DOMS symptoms after resistance exercise. which shows that the CTTM after the experiment in the experimental group, VAS was lower than the control group and increased range of motion.

Keywords: Court-Type Thai Traditional Massage (CTTM), delayed onset muscle soreness (DOMS), Biceps brachii

Introduction

Delayed onset muscle soreness (DOMS), also known as muscle fever, is a type of muscle strain injured is resulted in muscles had tenderness and stiffness. Most of the time, this condition occurs asymptotically, and tenderness tends to occur in the extremities of the muscles. The muscle pain receptors are located at the junction of muscles and tendons; difficulty moved pain occurs only when moved. The pain was present for the first 8-24 hours and the pain worsened after 24-72 hours after the exercise. Will had pain when touched the area.

There is stiffness, swelling and muscle weakness, etc. Symptoms last up to 96 hours (4 days) or more, and disappeared on their own, after 5-7 days of exercised is stopped depended on individual performance [1]. Treatment and prevention of DOMS, such as cold therapy with ice compress it would help reduced the inflammatory processed and swelled [2]. Stretched by warming up before exercise. Increased muscle flexibility and jointed range of motion would have a positive effect on reduced muscle spasm and increased blood flow [3]. Sports massage is popularly applied to

pained of DOMS is the Swedish massage used petri technique. Petrissage technique because it is a technique in the manner of pressed and released (Compression & Decompression) accorded to the pressure given to reduced pained swelled that occurs and increased blood flow, anti-inflammatory drug, ultrasound, electrical current techniques and homeopathy [4].

Court type Thai traditional massage (CTTM), Thai named Nuad Raja Sum Nak, is a practice of Thai traditional medicine. CTTM is a wisdom that has been passed down for a long time. Especially the group of diseases related to muscles, the massage used fingers and hands to massage the body according to the science and art of Thai traditional medicine that had been practiced in CTTM for therapeutic, disease prevention, rehabilitation. Helps relax muscles. This would be a good result in the treatment of pain, stiffness, fatigue in various parts of the body, reduced pain and swelling, etc. The benefits of CTTM, such as help the muscles relax and reduces muscle spasms, reduce muscle swelling, helps weaken the fascia, making the muscles more flexible and help to relax and relieve stress [5]

Therefore, the researcher is interested in studying the effects of CTTM on DOMS in the Biceps brachii muscle after resistance exercise because there are no research studies in this area. To be a guideline to fully rehabilitate the muscles after the next exercise as well as the integration of Thai traditional medicine in order to further disseminate wisdom.

Methodology

Participants

This study was approved by the Mae Fah Luang University Ethics Committee on Human Research (NO.EC-22029-25). This study was a clinical trial. This was a randomized compared study with a randomized control trial. Mae Fah Luang University student female, ages of 18-20 years, 30 people. The number of subjects was determined by a G*Power program, and the t-tests group data entry model was analyzed by means of the difference between two independent means (two groups). The effect size (effect size f) calculated from the above study was 1.09, which determined a confidence level of 95% and a power of the test (power of test) 80%. Total sample size: 30 people.

Inclusion Criteria

- Mae Fah Luang University students female, aged between 18-22 years
- Do not exercise regularly about using the arm area.
- Never received massage therapy before for at least 1 month.
- There are no contraindications to massage, fever above 38.5 degrees Celsius, high blood pressure of more than 140/90 millimeters points.

Exclusion Criteria

- The volunteer had symptoms related to disease in the jointed tendon or muscle which affects the inability to exercise according to the prescribed program.
- The volunteer was injured during the exercise program.

Experimental Design

This study was a clinical trial. This was a randomized compared studied with a randomized control trial. The volunteers were divided into 2 groups, the first group was the CTTM experiment and the second group was the control group the details are as follows. The first group, the experimented group, received a CTTM. The duration of the massage is 30 minutes. The second group was the control group. The volunteers were given bed rest and self-cared education for anterior forearm pain after resistance exercised.

The subjects tested the Visual Analogue Scale (VAS), Range of Motion (ROM) and swelling were measured at before the experiment, immediately and 24, 48 and 72 hours after the exercise.

Exercise

The armed training Scott-curl program causes muscle soreness on Biceps brachii. All volunteers performed eccentric exercise of biceps brachii muscles using a preacher curl bench. Weight of the dumbbell was 80% of the pre-exercise MIF. Place the upper arm on the cushion wider than your shoulders. By placing the elbows against the cushion. The volunteer lift the dumbbell from the elbow elastic to the elbow extension position and holds it for 5 seconds. Repeat 20 times, count as 1 set, do 3 sets.

Statistical Analysis

All analyses were performed using program SPSS for windows. Mean and the standard deviation (SD). Paired t-Test to compare the difference of the test resulted between the groups. Repeated measured to compared the differences of the test resulted within the group. Test for statistical significance at the 0.05 level.

Results

In a study on the Effect of Court-Type Thai Traditional Massage on Delayed-Onset Muscle Soreness (DOMS) in The Biceps Brachii Muscle After Resistance Exercise. A total of 30 volunteers were tested in both groups, including the Court type Thai Traditional massage group and the control group, 15 people each, as shown in table 1.

Table 1 General characteristics of participants

Volunteer attributes	Experimental group (n=15)	Control group (n=15)	P- value
Age (years)	20.80±0.91	21.10±1.10	0.39
Weight (kg)	58.85±8.48	57.30±10.70	0.66
Height (cm)	159.85±4.42	158.90±5.32	0.83

The VAS scores of the experimental group and the control group after massage (48 hrs.) were significantly different ($p < 0.05$) as shown in figure 1.

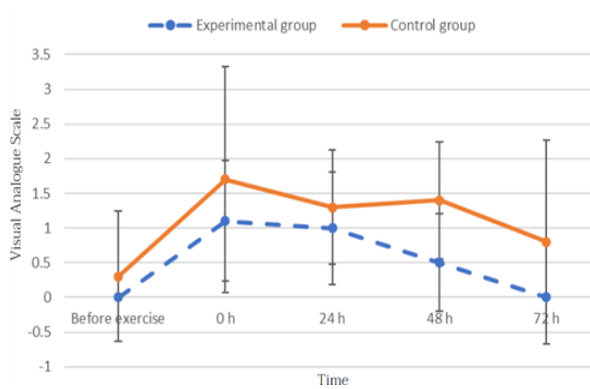


Figure 1 Comparison of VAS scores between experimental and control groups, $*(p < 0.05)$.

Range of motion (ROM) values between the experimental group and the control group after massage there were no significant differences as shown in figure 2.

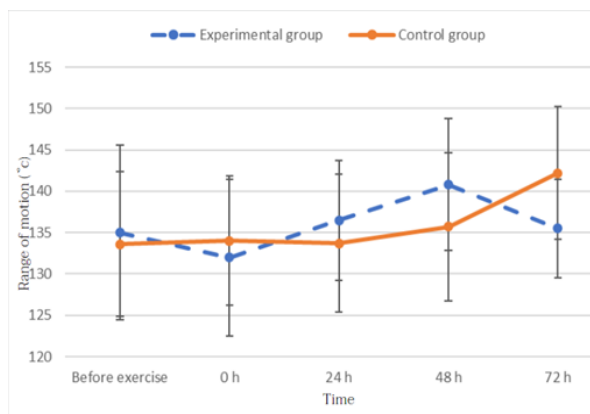


Figure 2 Comparison of ROM between experimental and control groups.

Swelling between the experimental group and the control group (72 hrs.) were not significantly different as shown in figure 3.

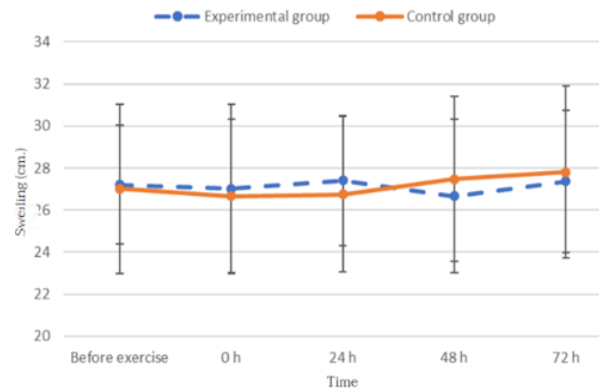


Figure 3 Comparison of Swelling between experimental and control groups.

The pain level of the experimental group and the control group showed that after massage VAS of the two groups there was no difference within the group, as shown in table 2. The ROM of elbow flexion between experimental and control groups. It was found that after massage ROM of both groups was not statistically different and there was no difference within the group. The range of motion of the experimental group's elbow flexion was increased after massage, as shown in table 3. The swelling of muscle between the experimental group and the control group found that the swelling after massage in both groups There were no statistically significant differences within and between groups, as shown in table 4.

*

Table 2 Comparison of VAS values within the experimental and control groups

Period	Pain Scale (VAS)				
	Before	After			
		0 hr	24 hr	48 hr	72 hr
Experimental group (n=15)	0.00±0.00	1.10±0.87	1.00±0.81	0.50±0.70	0.00±0.00
Control group (n=15)	0.30±0.94	1.70±1.63	1.30±0.82	1.40±0.84	0.80±1.47

Table 3 Comparison of ROM of elbow flexion within the experimental and control groups

Period	Range of motion (ROM)				
	Before	After			
		0 hr	24 hr	48 hr	72 hr
Experimental group (n=15)	135.00±10.54	132.00±9.48	136.50±7.26	140.80±8.01	135.50±5.98
Control group (n=15)	133.60±8.73	134.00±7.84	133.70±8.35	135.70±8.99	142.20±8.05

Table 4 Comparison of circumference length within the experimental and control groups

Period	Arm circumference length				
	Before	After			
		0 hr	24 hr	48 hr	72 hr
Experimental group (n=15)	27.21±2.84	27.40 ±3.10	27.37±3.38	27.53±2.87	27.44±3.36
Control group (n=15)	27.01±4.02	26.66±3.66	26.74±3.69	27.48±3.94	27.81±4.07

Discussion

VAS of the experimental group and the control group. There was a statistically insignificant reduction. When comparing the differences between the groups, it was found that There was a statistically significant decrease in pain level ($p < 0.05$) at 48 hours. ROM of the experimental group there was a statistically significant increase ($p < 0.05$) at 72 hours. And the control group When comparing the differences between the groups, it was found that There were no statistically significant differences. In addition, the swelling of the muscles There was no statistically significant in both group and between groups. The mechanisms of massage affect DOMS will reduce emigration of neutrophils and/or higher levels of serum cortisol [6]. Massage therapy after strenuous exercise could be effective for alleviating DOMS and improving muscle performance [7].

Conclusion

CTTM can help muscles relax, reduce muscle contraction and reduce DOMS symptoms after resistance exercise. Which shows that the CTTM after the experiment in the experimental group, the pain level was lower than the control group and increased range of motion. Future studies should include an exercise program that induces the whole arm and should increase the sample size. To support that CTTM can relieve DOMS symptoms after resistance exercise.

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Author contributions

Conceptualization and methodology: C.V., L.K., K.S. and B.S. Visualization: L.K., K.S. and B.S. Formal analysis: C.V., S.K. and T.T. Writing-original draft preparation: C.V., S.K. and T.T.

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Effect of Aromatic Medicine (YA HOM TEP PRA CHIT) on Patients with Hypertension

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ABSTRACT

Introduction: Aromatic medicine, using herbal remedies to help decrease blood pressure. There is a formulation that helps to treat lower blood pressure. It has properties to decrease blood pressure levels and nourish the heart called “YA HOM TEP PRA CHIT.”

Objective: The main objective of this study is to determine if adding aromatic medicine can decrease blood pressure with standard treatment for a patient with hypertension compared to standard treatment.

Methods: Study on the patient with hypertension in Wiang Chiang Rung Hospital. After purposive sampling, 80 patients were divided into 2 groups. The 40 people in the intervention group were treated by Select subjects based on screening with inclusion and exclusion criteria and were randomized into 2 groups such as the experimental group of patients taking Enalapril 5-15 mg /day) with Taking a dose of YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) once a day in the morning by the standards and evaluation satisfaction after the treatment

Results: The results of the study concluded that the average blood pressure at the pretest and post-test in the experimental group had an average decrease. Baseline characteristics between the treatment and control groups were similar. Before the treatment, the systolic blood pressure of the intervention and control group in term of after the treatment showed the average systolic blood pressure of the control group before joining the study and before the treatment are 153.13,153.83,153.93,154.98, and 154.98. The average diastolic blood pressure of the intervention group before joining the study and before the treatment showed the average diastolic blood pressure of the control group before joining the study and before the treatment are 84.38,85.05,85.53,86.15, and 86.40.

Conclusion: For the comparison of average systolic blood pressure after the treatment of both groups are statistically significant differences at the 0.05 level, but before the experiment, the average systolic blood pressure of both groups does not have statistically significant differences at the 0.05 level. And for the comparison of average diastolic blood pressure after the treatment in week2-4 of both groups are statistically significant differences at the 0.05 level.

Keywords: YA HOM TEP PRA CHIT; blood pressure; hypertension

Introduction

Hypertension is a major public health problem in Thailand. Currently, several changes cause changes in behavior. The hustle of life affects your health and body, causing even more. High blood pressure can cause many

complications. The most common complication of hypertension is heart coronary artery disease, kidney disease, kidney failure, and other complications. The World Health Organization survey and data collection of hypertensions around the world found that the death rate up to 12.8% of all mortality, or approximately 7.5

million people per year, and in May will have a patient population of 2025 is hypertension increased 1.2-1.5 billion people.[1] 2 and 3 in the pathogenesis of hypertension occur in developing countries in East Asia and Southeast today found the incidence of the disease is 1 in 3 people will suffer from high blood pressure. In Thailand, the number of patients with hypertension who registered more year, in 2017 the population of patients with hypertension registered an increase of 12,342.14 14,926.47 per 100,000 population and in Region 1 healthy body, including Chiang Mai, Lamphun, Phayao, Mae Hong Son and Chiang Rai, Phrae, Nan certain rate of patients with hypertension were 94,408 new cases in which the offense itself [2]. Chiang Rai has a new 1565.79 cases per 100,000 people in the year 2011. Department of Non-Communicable Diseases Department of Disease Control survey of high blood pressure both male and female, aged 15-74 years, hypertension often occurs in males: female ratio was 1.7: 1, most commonly between the ages 60 and over, followed by the age range of 40-59 years and an age range 15-39 years.[3] The severity classification of hypertension among adults aged 18 years and over was based on blood pressure levels measured in clinics, hospitals, or public health facilities. Hypertension care in Thailand: best practices and challenges, 2019. World Health Organization. Country Office for Thailand. Optimal systolic blood pressure < 120 and diastolic blood pressure < 80, Normal 120-129 and/or 80-84, High normal systolic blood pressure 130-139 and/or diastolic blood pressure 85-89, Hypertension level 1 systolic blood pressure diastolic blood pressure 140-159 and/or 90-99, Hypertension level 2 systolic blood pressure diastolic blood pressure 160-179 and/or 100-109, Hypertension level 3 systolic blood pressure diastolic blood pressure 180 and/or 110 and isolated systolic hypertension (ISH) systolic blood pressure diastolic blood pressure 140 and < 90. Treatment of hypertension is often mainly used to treat the medicine, patients taking drugs to lower blood pressure levels. This treatment is only for the good of the patient's symptoms are not worse than they were. The doctors have to learn to treat high blood pressure by focusing on behavior modification, controlling diet weight (Level of BMI) within the normal range, exercise, and mental health treatment. Otherwise, there is also hypertension treatment by using massage to help reduce blood pressure levels and remain at or below 130/85 mm Hg. to prevent complications and symptoms that may occur in the future.[4] Previous research has studied this quasi-experimental research Clinical practice guideline in Thai Traditional medicine for patients with hypertension in Chiang Saen Hospital 2014 showed that the treatment of hypertension can lower blood pressure as the systolic and diastolic equivalent of treating hypertension with medicine today [5,6] and education study massage therapy for essential

hypertension: a systematic review showed that massage may play a critical role in controlling high blood pressure in the treatment of primary support therapy, when combined with high blood pressure, may be more effective than drugs for high blood pressure as well as reductions in systolic blood [7] and a study of the effect Massage therapy for essential hypertension: a systematic review showed that massage may play a critical role in controlling high blood pressure in the treatment of primary support. Massage therapy, when combined with high blood pressure, may be more effective than drugs for high blood pressure as well as reductions in systolic blood pressure and a study of the effect of Massage therapy for essential hypertension: a systematic review showed that massage may play a critical role in controlling high blood pressure in the treatment of primary support. Massage therapy, when combined with high blood pressure, may be more effective than drugs for high blood pressure as well as reductions in systolic blood pressure.[7] From the above, you can see that taking a dose of YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) may help lower the blood pressure of patients to come back in the stable or less than the original. Thus, the authors of the research promote the use of traditional medicine in Thailand to treat patients with high blood pressure further. Researchers are interested to study the efficacy and safety of the YA HOM TEP PRA CHIT formula that can decrease blood pressure with standard treatment on a patient with hypertension and compare patients with hypertension with standard treatment.

Methodology

The study design was Quasi-Experimental research that compared the intervention and the control group by collecting data from a sample group of male or female patients aged 34-59 years. After purposive sampling, 80 patients were divided into 2 groups 40 people each based on the criteria of the inclusion criteria as has been diagnosed with essential hypertension or primary hypertension and receiving high blood pressure treatment by taking Enalapril 5-15 mg. once a day in a morning by the standards of the Wiang Chiang Rung Hospital Chiang Rai, blood pressure ranges from 140/90 mmHg but not exceeding 160/100 mmHg, not have dyspnea/chest pain/pulse rate more than 100 times/minute and irregular pulse, no complications as a result of high blood pressure to the target organs such as the kidneys or the heart and so on, and no other common diseases such as high blood lipid or diabetes, asthma, heart disease, cancer, stroke and renal disease based on the history of treatment, and pleased to cooperate in this study. The two groups signed the consent form to participate in the study. Discontinuation criteria are blood pressure is greater than or equal to 160/100 mmHg

From blood pressure monitoring to 2 rounds[8] (according to the schedule of activities), found symptoms of cardiovascular disorders such as chest pain, etc., or signs of urinary tract disorders, such as urinary incontinence, bloody urine, etc., found a disease that occurred during the research was diagnosed as life-threatening if still in the research stage and not treated, do not follow the rules of research or not follow the schedule, and want to withdraw from this study. The 40 people in the intervention group were treated such as the experimental group Patients taking Enalapril 5-15 mg /day) by the standards for the treatment of hypertension in early-stage patients, the dose of Enalapril was able to control blood pressure due to its 24 -hour duration by taking a dose of YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) once a day in a morning by the standards of the Wiang Chiang Rung Hospital Chiang Rai, and evaluation satisfaction after the treatment at week4.

Statistical analysis

The study duration is 8 weeks containing the steps for preparation, clinical examination, and collecting data. The steps for preparation. Study documents and related research. Contact the relevant authorities and gather the required information. Procedures for ethical research are the step for clinical examination and collecting data. Choose population and clinical examination process. The step for process and analyzing the data. Explain statistics of variable data by mean percentage and standard deviation. Use independent sample t-test and SPSS program to compare mean between experimental group and control group. All statistical tests were performed at a two-sided p-value less than 0.05 levels.

Results

This research is quasi-experimental research to study the effect of high blood pressure treatment by an experimental group of patients taking Enalapril 5-15 mg /day) taking a dose of YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) with standard treatment and control group taking standard treatment and taking Enalapril 5-15 mg. Once a day in the morning. Measurement of blood pressure and side effects and also compared the satisfaction of those who

received high blood pressure treatment at Wiang Chiang Rung Hospital, Wiang Chiang Rung District, and Chiang Rai province. The research team collected data from 80 patients, divided into 40 experimental groups and 40 control groups. The data was analyzed and presented in the form of a lecture table divided into 3 parts.

Part 1: General characteristics of the sample

Personality

The general characteristics of the sample show the characteristic of the sample group. The most gender in the sample group is female; the experimental group is 60.00 percent and the control group is 65.00 percent. The most age in the experimental group is 50-54 and 55-59 years old with 40.00 percent. Then the second is 45-49 and 40-44 years old with 7.50 percent the most age in the control group is 50 -54 years old with 32.50 percent. The most Marital status is married which has the same in 2 groups. Married in the experimental group has 85.00 percent and, in the control, the group has 80.00 percent.

Part 2: The result of the treatment of hypertension by YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) in combination with taking Enalapril 5.15 mg. per oral once a day in the morning.

From table 1, the comparison of the result of the treatment by YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) in combination with taking Enalapril 5-15 mg. per oral once a day in the morning of the intervention and the control group in term of before the treatment showed that an average of systolic blood pressure of the intervention group before joined the study and before the treatment in week1, week2, week, and week4 are 151.50, 138.40, 133.48, 130.83 and 129.93. The average systolic blood pressure of the control group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 were are 153.13, 153.83, 153.93, 154.98, and 154.98. The average diastolic blood pressure of the intervention group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 are 84.23, 83.78, 81.53, 80.78, and 81.28. The average diastolic blood pressure of the control group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 are 84.38, 85.05, 85.53, 86.15, and 86.40.

Table 1 Showed an average, standard deviation and compares the average blood pressure of the intervention group (N = 40) and the control group (N = 40) before joining the study and before the treatment in week1, week2, week3, and week4 divide into systolic and diastolic

Blood Pressure		Blood Pressure (mmHg) Intervention group (N=40)		Blood Pressure (mmHg) Control group (N=40)	
		Mean	SD	Mean	SD
Systolic	Before	151.50	12.77	153.13	11.67
	Week-1	138.40	17.09	153.83	10.84
	Week-2	133.48	15.93	153.93	10.58
	Week-3	130.83	14.15	154.98	10.30
	Week-4	129.93	12.52	154.98	10.30
Diastolic	Before	84.23	12.07	84.38	11.82
	Week-1	83.78	14.80	85.05	11.54
	Week-2	81.53	17.51	85.53	11.48
	Week-3	80.78	13.02	86.15	11.26
	Week-4	81.28	12.04	86.40	11.46

Table 2 Showed an average standard deviation and compares the average blood pressure of the intervention group (N = 40) and the control group (N = 40) after the treatment in week 1, week 2, week 3, and week 4 divided into systolic and diastolic.
 Note:*Statistical significance at the 0.05 level.

Blood Pressure	Blood Pressure (mmHg)		t	df	P-value
	Mean	SD			
Before					
Intervention group Systolic	151.50	12.77	-.594	78	0.554
Control group Diastolic	153.13	11.67			
Intervention group Systolic	84.23	12.07	-.056	78	0.955
Control group Diastolic	84.38	11.82			
Week1					
Intervention group Systolic	138.40	17.09	-4.820	78	0.000*
Control group Diastolic	153.83	10.84			
Intervention group Systolic	83.78	14.80	-.430	78	0.669
Control group Diastolic	85.05	11.54			
Week2					
Intervention group Systolic	133.48	15.93	-6.760	78	0.000*
Control group Diastolic	153.93	10.58			
Intervention group Systolic	81.53	17.51	-1.208	78	0.231
Control group Diastolic	85.53	11.48			
Week3					
Intervention group Systolic	130.83	14.15	-8.618	78	0.000*
Control group Diastolic	154.98	10.30			
Intervention group Systolic	80.78	13.02	-1.974	78	0.052
Control group Diastolic	86.15	11.26			
Week4					
Intervention group Systolic	129.93	12.52	-9.768	78	0.000*
Control group Diastolic	154.98	10.30			
Intervention group Systolic	81.28	12.04	-1.949	78	0.055
Control group Diastolic	86.40	11.46			

From table 2 the comparison of the result of the treatment by YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) in combination with taking Enalapril 5-15 mg. per oral once a day in the morning of the intervention and the control group in term of after the treatment showed that 151.50,138.40,133.48,130.83 and129.93. The average systolic blood pressure of the control group before joining the study and before the treatment in week 1, week2, week3, and week4 is 153.13, 153.83, 153.93,

154.98, and 154.98. The comparison of average systolic blood pressure after the treatment in week-1,2,3 and week-4 of both groups are statistically significant differences at the 0.05 level, but before the experiment, the average systolic blood pressure of both groups is not statistically significant differences at the 0.05 level. The average diastolic blood pressure of the intervention group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 are 84.23,83.78,81.53,80.78, and 81.28. The average of

diastolic blood pressure of the control group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 are 84.38,85.05,85.53,86.15, and86.40. For the comparison of average diastolic blood

Discussion

The results of the study concluded that the experimental group's average blood pressure at pretest and post-test had an average decrease. Baseline characteristics between the treatment and control groups were similar. Before the treatment, the systolic blood pressure of the intervention and control group is the comparison to the result of the treatment by YA HOM TEP PRA CHIT 3 capsules 3 times before meal (1,200 mg/day) in combination with taking Enalapril 5-15 mg. per oral once a day in a morning of the intervention and the control group in term of after the treatment showed that 151.50,138.40,133.48,130.83 and129.93. The average of systolic blood pressure of the control group before joining the study and before the treatment in week 1, week 2, week 3, and week 4 were are153.13, 153.83, 153.93, 154.98, and 154.98. The comparison of average systolic blood pressure after the treatment in week-1,2,3 and week-4 of both groups are statistically significant differences at the 0.05 level, but before the experiment, the average systolic blood pressure of both groups is not statistically significant differences at the 0.05 level. The average of diastolic blood pressure of the intervention group before joining the study and before the treatment in week1, week2, week3, and week4 are 84.23,83.78,81.53,80.78, and 81.28. The average diastolic blood pressure of the control group before joining the study and before the treatment in week1, week2, week3, and week4 are 84.38,85.05,85.53,86.15, and86.40. For the comparison of average diastolic blood pressure after the treatment in week3 and week4 of both groups are no statistically significant differences at the 0.05 level. The effect of YA HOM TEP PRA CHIT on the circulatory system of the heart, brain, and throughout the body, which was discovered from modern research as evidence confirming the properties of aromatherapy. It has been found since ancient times that it helps to relieve palpitations, fainting, blackouts, dizziness, and dizziness. Beriberi, including symptoms due to low blood pressure and insufficient blood supply to the brain, resulting in increased blood flow of small blood vessels to the brain and peripheral organs of the entire body and organs outside the body, such as blood vessels that feed the hands, feet, etc. However, the use of YA HOM TEP PRA CHIT Should be used according to the indications and precautions as follows should be careful when eating with drugs in the anticoagulant group (anticoagulant) and antiplatelet drugs (antiplatelets) and

pressure after the treatment in week3 and week 4 of both groups are no statistically significant differences at the 0.05 level.

be careful with continued use of the drug. Especially in patients with hepatobiliary dysfunction. Because camphor accumulation and poisoning may occur and be careful with the drug in patients allergic to pollen.

Conclusion

This is consistent with the research on the treatment of high blood pressure by Court-type Thai traditional such as medical massage exercise combined with modern medicine can reduce blood pressure while heart compression and relaxation as well treatment of high blood pressure with modern medicine.[9] This is consistent with Chairat N.'s research that compares the treatment of hypertension between Court-type Thai traditional and modern medicine which compares the average systolic and diastolic blood pressure before and after the treatment in week4, week8, and week12 [5,10] of the intervention group and control group and the mean was significantly lower at 0.001. The comparison in each week shows that the mean was slightly decreased. The decrease is statistically significant at 0.05 levels. YA HOM TEP PRA CHIT formulation capsule was well tolerated. No clinically meaningful difference in the overall adverse event. There was no serious clinical adverse event. Frequent urination and diarrhea after taking medication were reported in both groups. For this reason, we are unable to make a definite conclusion about the effectiveness of the YA HOM TEP PRA CHIT formulation capsule. However, the results of this study can be used to estimate the sample size for bigger studies. In conclusion, the treatment of high blood pressure by YA HOM THE PRA CHIT can reduce blood pressure levels statistically significantly compared with modern medicine's treatment. Therefore, Thai traditional medicine should be applied to treat patients with hypertension combined with modern medical treatment.

Competing Interests

The authors declare no conflict of interest. The funders had no role in the design of the study in the collection, analysis, or interpretation of the data in the waiting of the manuscripts, or in the decision to publish the result.

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Author contributions

Conceptualization and methodology: T.T., and K.S. Visualization: T.T., K.S., and C.V. Formal analysis: T.T., K.S., and C.V. Writing-original draft preparation: T.T.

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The Effectiveness of Thai Massage on the Range of Motion and Muscular Strength of the Arm in Collegiate Basketball Players, Mae Fah Luang University

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ABSTRACT

Introduction: Basketball is a fast pace sport, where the players are required to move quickly, catch and shoot the ball. Basketball players have been developed various techniques using upper limbs such as shooting, throwing, passing, and dribbling. In addition to exercise training for high level of physical fitness, sports massage with stimulating technique is also using for the short-term effectiveness.

Objective: The purpose of this study was to determine the effectiveness of Thai massage on the range of motion (ROM) of the right shoulder and muscular strength of the right arm in the collegiate basketball players.

Methods: Twenty subjects, aged from 19 to 24 years old, were equally randomized into two groups: Thai massage group was treated with Thai Massage program and control group was treated with Swedish massage program for 10 minutes. Shoulder ROM and grip strength were measured immediately before the intervention, and after the intervention. The duration of the trial was 2 times, with the first trial - two days off - the second trial.

Results: The results showed that Thai massage program improved range of motion of the right shoulder. Data showed a significant difference in shoulder abduction ($p < 0.05$) and adduction ($p < 0.05$) compared with control group. Additionally, Thai massage program significantly promoted short-term effect on muscular strength of the right arm ($p < 0.05$) compared with control group in 2 trials.

Conclusion: These results suggested that the Thai massage program has an short-term effects on the range of motion of the right shoulder and muscular strength of the right arm, compared to Swedish massage in the control group.

Keywords: Range of motion, Muscular strength, Thai massage, Swedish massage

Introduction

Basketball is about scoring goals and scoring. The goal is achieved by shooting the ball through the opponent's basket or hoop using the muscles in the arm. Points are awarded by throwing the ball into the hoop from above. Basketball has developed various playing techniques such as shooting, passing, and dribbling, including the player position for athletes who are equipped with physical fitness and skilled performance, that person has a very good chance of winning against

their opponents. This sudden movement requires the control and coordination of the neuromuscular nervous system (Coordination) as well, so it will be accurate and fast (Speed). In addition, sports massage with a quick and stimulating technique is also the preferred method used by athletes before the competitions or training sessions. Exercising and playing sports is the coordination of all muscles and when working repeatedly for a long time. The body will become fatigued, which causes the body's nervous system to be

impaired in order to maintain the muscles and unable to build. The energy in a steady-state (steady-state) if the athlete has a complete physical preparation will have a greater chance of winning in that competition, which sports massage is widely used. At present, both the leading coaches in fitness gyms including athletes [1,2] report that sports massage can be divided into 3 types: pre-match massage. Massage for most athletes, Swedish massage is a form of massage that is universal and is accepted all over the world in principle, Swedish massage is a massage of touch, press, squeeze and touch the different parts of the body using the principle. The same massage as Thai massage is a massage to relax the mind and body for comfort and healing. A study [3] compared Swedish massage and Thai massage in physiology and psychology. They were randomly assigned to receive either Thai massage or Swedish massage. Dependent variables were blood pressure and heart rate, range of motion. Awareness of Anxiety and Emotions The physical assessment was assessed 48 hours before and after the massage. The results showed that Thai massage and Swedish massage were equally effective. The benefits of massage for athletes are to increase blood flow, expand blood, increase the amount of blood that the heart pumps out, reduce the pulse rate increase the readiness of body Affects the stimulation, causing the maximum energy of the muscles to increase [4,5], can relieve muscle tension. and allows the connective tissue to be more stretched since massage helps to stretch or relax the fascia muscles. Ligaments that hold bones and muscles which helps to increase the backflow of the veins and increase the flow of arteries to replace more Histamine is released causing blood vessels to dilate. increased skin temperature resulting in the excretion of waste products from various metabolic processes including lactic acid (lactic acid) faster. At the same time, it helps to increase the supply of oxygen and nutrients to the tissues. It helps to heal and reduce muscle spasms as well. There is a drain or a reduction in the amount of various pain neurochemicals. born in the injured area Reduces the stimulation of small nerve fibers Affects the recovery of muscle function after exercise. Relaxation helps the athlete's body to return to normal faster [6]. Massage during the match and post-match massage, where the pre-match massage is a stimulating massage on the superficial tissue with fast strokes with light weights, use common tech, avoiding deep, heavy strokes, or press only the joints. This type of massage can be performed three days before the match up to four hours before the match. According [7], it was found that regular stretching of muscles improves mobility and prevents injuries. In addition, if an athlete develops a good level of flexibility, it will improve the technical skills that athletes need to use force, agility, and increased mobility. In this study, research was conducted to apply the results of the research to athletes to optimize arm muscle strength before training and before the competition. The purpose of this study wants

to study the effectiveness of Thai Massage on the range of motion of the right shoulder and muscular strength of the right arm in collegiate basketball players.

Methodology

Participants

The research design in this study is clinical research conducted as a controlled trial. Researchers will recruit participants who are male, undergraduate students who are basketball players under the Basketball Club of Mae Fah Luang University, aged 19 to 24, 20 participants. The participants were in good health and have not a history of serious diseases such as bone cancer, arm vein thrombosis, diabetes, etc., or had serious injuries to the muscles and ligaments at the shoulder for at least six months, must have similar physical fitness and must have a 2-day muscle break. During the trial, the experimenter had an accident such as an injury to the muscles, bones, and joints of the arm. The participants can leave the experiment at any time.

Methods

Participants who passed the criteria will be informed about the research objectives and the protocol. After they listen to the orientation, all of them must give verbal consent and sign their written consents to this study. They are also informed that they can drop out any time they wish even before they complete the trial. Participants were divided into two groups: an experimental group of 10 people and a control group of 10 people, by choosing sealed envelopes. A lottery was drawn, using code 01 as a basketball player group receiving a Thai massage program and 02 as a basketball player group receiving a Swedish massage program. The researcher will collect the information before and after these participants receive the Thai massage program and participants receive the Swedish massage program by measuring the range of motion of the shoulder with a goniometer, a push-pull dynamometer; measuring muscle strength with a Handgrip Dynamometer. Experimental group, participants who received a Thai massage program will receive a Thai massage two times, with the first trial - two days off - the second trial. Use the thumb and hands to massage the body area and apply 50 pounds of force for massage. a Thai massage program had an inner arm massage, a wind gate point, and an outer arm massage, the total massage time is 10 minutes. Only the right arm. Control group, participants who received a Swedish massage program will receive a Swedish massage two times, with the first trial - two days off - the second trial. Use the hands to massage the body area and apply light force for massage. A Swedish massage program had effleurage, petrissage, friction, vibration, and tapotement. The total massage time is 10 minutes.

Statistical Analysis

The results are shown in terms of the standard deviation (S.D.) for the Shoulder range of motion (ROM) and muscular strength of the right arm by using an SPSS program. And compare the results of the Thai massage program, the repeated measures ANOVA statistic was used as a mean score difference between the experimental group and the control group. Including the difference in the experimental group.

Results

The sample group participating in this research study were 20 male students of Mae Fah Luang University, divided into the group receiving Thai massage and a control group of 10 people each. The experimental group has an average age of 21.80 ± 1.13 years and an average weight of 73.70 ± 10.12 kg. average height 175.50 ± 4.64 cm. The control group has an average age of 21.20 ± 0.63 years and an average weight of 67.70 ± 2.40 kg. average height 173.40 ± 6.04 cm

Table 1 Basic information of the experimental and control groups show average and standard deviation.

Basic information	The experimental group (n = 10)	The control group (n = 10)
Age (year)	21.80±1.13	21.20±0.63
Weight (kg.)	73.70±10.12	67.70±2.40
Height (cm.)	175.50±4.64	173.40±6.04

The average muscular strength of the right arm in the experimental group. Pretest, first post-test, and second post-test were 84.39, 89.25, and 93.99, respectively. In the control group. Pretest, first post-test, and second post-test were 76.24, 82.38, and 87.12, respectively. The average range of motion of the right shoulder, Rt.

Shoulder adduction (°) in the experimental group. Pretest, first post-test, and second post-test were 47.00, 47.60, and 47.20, respectively. In the control group. Pretest, first post-test, and second post-test were 47.10, 48.20, and 49.20, respectively.

Table 2 The results of the comparison of the average range of motion of the right shoulder and the muscular strength of the right arm of the experimental group and control group.

Variable	Experimental			Control		
	Pre	Post1	Post 2	Pre	Post 1	Post 2
Rt. Hand grip (lb.)	84.39	89.25	93.99	76.24	82.38	87.12
Rt.Shoulder adduction (°)	47.10	48.20	49.20	47.00	47.60	47.20
Rt.Shoulder abduction (°)	176.60	178.40	178.60	177.70	178.60	178.80

From table 3 The muscular strength of the right arm between the experimental group and the control group. Before receiving Thai massage in the experimental group and receiving Swedish massage in the control group, there was a statistically significant difference ($p < 0.05$). The experiment and control groups were 84.39 ± 16.35 and 76.24 ± 10.32 , respectively, and the muscular strength of the right arm of the experimental group was higher than the control group. The average range of motion of the right shoulder, Rt. Shoulder abduction (°) in the experimental group. Pretest, first post-test, and second post-test were 177.70, 178.60, and 178.80, respectively. In the control group. Pretest, first post-test, and second post-test were 176.60, 178.40, and 178.60, respectively. The muscular strength of the right arm between the experimental group and control group.

After the first receiving massage, Thai massage in the experimental group, and Swedish massage in the control group. There was a statistically significant difference ($p < 0.05$). The experiment and control groups were 89.25 ± 18.68 and 82.38 ± 8.72 , respectively, and the muscular strength of the right arm of the experimental group was higher than the control group. The muscular strength of the right arm between the experimental group and control group. After the second receiving massage, Thai massage in the experimental group, and Swedish massage in the control group. There was a statistically significant difference ($p < 0.05$). The experiment and control groups were 93.99 ± 19.40 and 87.12 ± 8.88 , respectively, and the muscular strength of the right arm of the experimental group was higher than the control group.

Table 3 The results of the comparison of the average muscular strength of the right arm of experimental group and control group

Period	Rt. Hand grip (lb.) muscular strength	
	Experimental	Control
Pre	84.39 ± 16.35	76.24 ± 10.32
Post 1	89.25 ± 18.68*	82.38 ± 8.72
Post 2	93.99 ± 19.40*	87.12 ± 8.88

* Significant differences between the experimental group and the control group ($p < 0.05$).

From table 4 Range of motion of Rt. Shoulder adduction between Experimental group and Control group. Before receiving Thai massage in the experimental group and receiving Swedish massage in the control group, there was a statistically significant difference ($p < 0.05$). The experiment and control groups were 47.00 ± 3.09 and 47.10 ± 2.38 , respectively, and Rt. Shoulder abduction of the experimental group was lower than the control group. Range of motion of Rt. Shoulder adduction between Experimental group. After the first, receiving Thai massage, there was a statistically significant difference ($p < 0.05$), which was 48.20 ± 2.10 . Range of motion of Rt. Shoulder

adduction between Control group. After the first, receiving Swedish massage is not a statistically significant difference ($p < 0.05$, were 49.20 ± 1.03 . And Rt. The shoulder adduction of the experimental group was lower than the control group. Range of motion of Rt. Shoulder adduction between Experimental group and Control group. After the second receiving massage, Thai massage in the experimental group, and Swedish massage in the control group. Is that not a statistically significant difference ($p < 0.05$). The experiment and control groups were 47.60 ± 2.63 and 47.20 ± 3.42 , respectively, and Rt. The shoulder abduction of the experimental group was lower than the control group.

Table 4 The results of the comparison of the average range of motion (ROM) of the right shoulder, Rt. Shoulder adduction of experimental group and control group

Period	Rt. Shoulder adduction (°) range of motion (ROM)	
	Experimental	Control
Pre	47.10 ± 2.38	47.00 ± 3.09
Post 1	48.20 ± 2.10*	47.60 ± 2.63
Post 2	49.20 ± 1.03*	47.20 ± 3.42

* Significant differences between the experimental group and the control group ($p < 0.05$).

From table 5 Range of motion of Rt. Shoulder abduction between experimental group and control group. Before receiving Thai massage in the experimental group and receiving Swedish massage in the control group, there was a statistically significant difference ($p < 0.05$). The experiment and control groups were 176.60 ± 3.06 and 177.70 ± 1.95 , respectively, and Rt. The shoulder abduction of the experimental group was higher than the control group. Range of motion of Rt. Shoulder abduction between experimental group and control group. After the first receiving massage, Thai massage in the experimental group, and Swedish massage in the control group. There was a statistically

significant difference ($p < 0.05$). The experiment and control groups were 178.40 ± 2.76 and 178.60 ± 1.71 , respectively, and Rt. The shoulder abduction of the experimental group was higher than the control group. Range of motion of Rt. Shoulder abduction between experimental group and control group. After the second receiving massage, Thai massage in the experimental group, and Swedish massage in the control group. There was a statistically significant difference ($p < 0.05$). The experiment and control groups were 178.80 ± 1.62 and 178.60 ± 2.55 , respectively, and Rt. The shoulder abduction of the experimental group was higher than the control group.

Table 5 The results of the comparison of the average range of motion (ROM) of the right shoulder, Rt. Shoulder abduction of experimental group and control group

Period	Rt. Shoulder abduction (°) range of motion (ROM)	
	Experimental	Control
Pre	176.60 ± 3.06	177.70 ± 1.95
Post 1	178.40 ± 2.76*	178.60 ± 1.71
Post 2	178.60 ± 2.55*	178.80 ± 1.62

* Significant differences between the experimental group and the control group ($p < 0.05$).

Discussion

From research of the effectiveness of Thai massage on the range of motion and muscular strength of the arm in collegiate basketball players, Mae Fah Luang University. That the researcher has studied and gathered various opinions as well as related research results can be presented to discuss the results according to each hypothesis as follows: the range of motion of the right shoulder and muscular strength of the right arm of the experimental group, after receiving the Thai massage program was greater than the control group. From table 2 - 5 the results of the comparison of the average range of motion of the right shoulder and the muscular strength of the right arm of the experimental group and control group, there were significant differences in the range of motion of the right shoulder and muscular strength of the right arm of the experimental group and the control group. This is consistent with the research [8] found that Thai massage improves blood circulation. Massage also reduces muscle tightness, and the massage program causes the muscles to attach and relax the fascia muscles, tendons, bones, and muscles stretch and contract, there will be changes within the muscle at the sarcomere level in the capillary fibers. The sarcoma contracts and then relaxes stretches or returns, staying the same width [9] as a result of the conversion of chemical energy to mechanical energy. From this contraction, the whole muscle transmits a strong stream of mechanical energy to better contract the muscles. The result in movement is more efficient, known as the Stretch-shortening cycle [10]. A review of related research found that Thai massage helps muscles contract and relax, make the body have good movement. The range of motion of the right shoulder and muscular strength of the right arm of the experimental group after receiving the Thai massage Program in the 1st and 2nd time were not different from the control group. From table 2 Range of motion of the right shoulder and muscular strength of the right arm of the experimental group and control group in the 1st and 2nd time. Because the Thai massage program is only for the arms. Use a royal massage style. Using a massage pressure of 50 pounds means pressing a massage with a small weight or called a light weight, profoundly relaxing, this is consistent with the research [11] applied Thai massage. It is used for warm-ups that require intense muscle bursts. It was concluded that in the massage Each time has the same warm-up effect.

Conclusion

The research found the effectiveness of Thai massage on the range of motion and muscular strength of the arm by comparing the two massage sessions in collegiate basketball players, at Mae Fah Luang University. It was found that each time the movement of the range of motion and muscular strength of the arm, indicating that Thai massage program may have the effectiveness on the range of motion and muscular strength of the arm.

Competing Interests

The authors declare no conflict of interest. The funders had no role in the design of the study in the collection, analyses, or interpretation of the data in the waiting of the manuscripts or in the decision to publish the result.

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Author contributions

Conceptualization and methodology: T.T., and K.S. Visualization: T.T., K.S., and C.V. Formal analysis: T.T., K.S., and C.V. Writing-original draft preparation: T.T.

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Effect of Court-Type Thai Traditional Massage Versus *Cassia alata* Linn. Treatment on Chronic Constipation: A Randomized Controlled Trial

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ABSTRACT

Introduction: 24 percent of the Thai population thinks they have constipation, it was found that about 10 percent who had defecation less than three times a week or had difficulty defecation had different health effects. Abdominal pain or bleeding from the rectum in cases of hemorrhoids causing ulcers in the colon or around the anus. Constipation can also be a symptom of a serious illness, such as colon cancer. It can make you depressed, and socioeconomic consequences may cause the patient to be absent or unable to work fully.

Objective: This study aimed to compare Court-type Traditional Thai massage with the used *Cassia alata* drug to treat chronic constipation.

Methods: This study was a Randomized Controlled Trial, randomized 2-group trial, 30 patients were randomly allocated to use Court-type Traditional Thai massage and to use *Cassia alata* drug (15 patients/group). Patients in the massage group received 60 minutes sessions of Court-type Traditional Thai Massage 2 times a week and the *Cassia alata* drug used group received 1 sachet of drug dissolve warm water 7 days in a row (before bedtime). Functional constipation was measured before (pre-test) and after (post-test) 7 days compared between 2 groups by the constipation assessment tools, adverse reaction assessment form, and satisfaction assessment form.

Results: A study showed the massage group differed significantly from the drug group ($P < 0.05$). However, the adverse reaction assessment indicated no symptoms after massage while the drug user group had symptoms; of dizziness, nausea, and stomach discomfort after using the *Cassia alata* drug.

Conclusion: Court-type Traditional Thai Massage and Using *Cassia alata* drug was effective in treating functional constipation. However, massage therapy has no symptoms, and stomach comfort after treatment satisfies patients.

Keywords: court-type traditional Thai massage; *Cassia alata*; chronic constipation

Introduction

Constipation is a condition in which the frequency of defecation is less than usual. Or there may be a normal defecation frequency. But each defecation is difficult, with a normal person defecation from 3 times a day to 3 times a week. Therefore, people who defecate less than 3 times a week are considered constipated if

they do not defecate more than 3 times a week. days are referred to as chronic constipation [1]. It is a serious health problem that interferes with your well-being and can have serious consequences. It is a symptom that often does not receive much attention, despite the fact that constipation is common in both normal people and patients. A study on the incidence of constipation in

foreign countries Found about 16-17% of constipation and 24.2 percent in Thai people [2,3]. Causes of Constipation It comes from many reasons such as low fiber diet, stress and side effects from taking medications. inappropriate excretion habits [4]. which constipation in the science of Thai traditional medicine It is stated that it is the cause of Dan Lom disease, which is a type of disease that appears in Thai traditional medicine textbooks. This is caused by chronic constipation. and deterioration of the body [5], but which in the Scriptures (The scriptures that talk about the disease of the wind), which is caused by living in an area too hot or too cold [6]. Eating cold or hot food can be seen that according to each scripture there is a cause. Different pathogens represent different methods of treatment. Such diseases are more common. The predominant symptoms of flatulence are as follows: chronic constipation for more than 4 days, characterized by hard stools similar to goat feces, back pain, bad breath, dizziness, mood disturbances, colic, abdominal distension, as well as numbness of the hands and feet, and Skin itching in some cases [7]. When constipation occurs, it affects the patient in psychosocial aspects, discomfort, and suffering. suffering from constipation results in anxiety increased anxiety reduces the quality of life and economically, from both physical and mental impacts, resulting in the loss of the patient's expenses for self-care increases [8]. Currently, there are two methods for treating constipation, which are drug and non-drug treatment. Most of them use laxatives or douche. This is a solution to occasional constipation. and may result in negative consequences from the use of laxatives for a long time. This can cause bloating, nausea, diarrhea or drug resistance. It also makes his body dehydrated and mineral salt, including the risk of drug complications [8]. And non-drug treatment but using rehabilitation principles to stimulate the functioning of the organs associated with managing the underlying causes of constipation, such as stimulating bowel movements by adding 25-30 g of fruit and vegetables per day, stimulating the intestines by walking 30 minutes after a meal. or self-abdominal massage in those who cannot be able to walk and sit and defecate immediately after meals [9]. It has also been found that Thai massage improves the motility of the abdominal organs, cavities and intestines. Aids in defecation and reduces dependence on laxatives, which can cause complications of the systemic digestive system, is an effective alternative therapy to medication [10]. Although the effectiveness of Thai massage, especially abdominal massage, is known to be effective in relieving constipation. However, the efficacy of court-type Traditional massage in relieving constipation compared to drug treatment was limited [11,12]. The efficacies of court-type Traditional massage and *Cassia alata* Linn. treatment was assessed and compared in alleviating constipation. This research will contribute to

the development of Thai traditional medicine and the preservation of local wisdom, while the findings can be used as guidelines for the treatment of constipation and healthcare improvement.

Methodology

The present study was a single-blind randomized controlled trial using a simple random sampling method which a pre-generated random assignment scheme enclosed in an envelope by one of the authors.

Subject

This study was a clinical trial, experimental Study: Randomized Controlled Trial (RCT) was a comparative study on the effectiveness of Court-type Traditional Thai massage and *Cassia alata* drug on the treatment of chronic constipation in 2 groups use purposive sampling 15 subjects per group for this pilot study. Court-type Traditional Thai massage of 15 people and a control group (*Cassia alata* drug), 15 people, measured twice, before and after the experiment. Patients were eligible and had no contraindications for participation in the study will be randomly placed in either the experimental group or the comparison group by simple randomization by drawing method to get equal opportunities prior to the randomization, each patient was informed that they may have been treated either by Thai massage for constipation or had received *Cassia alata* in advance and were informed of the information they had to be involved in the research and were willing to sign. Consent to participate in the research following a pre-generated random assignment scheme enclosed in envelopes (Figure 1).

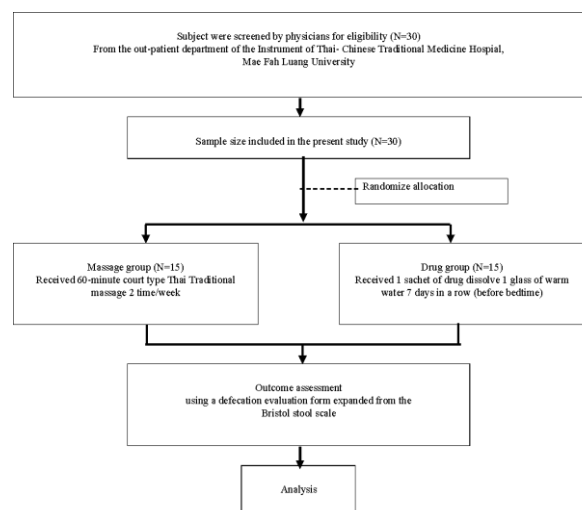


Figure 1 Consort flow diagram for patient selection

Inclusion and Exclusion criteria

Inclusion criteria were 1) Female or male aged 16-30 years, 2) has been diagnosed by a doctor for chronic constipation according to the Rome III Diagnostic

Criteria for Functional Gastrointestinal Disorders consist of 2 of the following: there must be a 25% strain on the stool during defecation, and the stool is lumpy or hard at 25% of the defecation, feeling that the defecation is inadequate at least 25% of the defecation, feeling that defecation is not possible as if something is blocking the anus for at least 25% of defecation, there must be assisted defecation at least 25% of the defecation (eg finger support, support of the pelvic floor), defecation less than 3 times weekly with soft stools are rare without the use of laxatives and do not meet the diagnostic criteria for irritable bowel syndrome, 3) there are no contraindications to massage, such as fever over 38.5 degrees Celsius, high blood pressure more than 140/90 mm. PT, skin diseases such as inflammatory wounds, infectious diseases such as tuberculosis, AIDS, brittle bones, osteoporosis, and herniated discs. Decline, 4) there were no contraindications for abdominal massage such as confusion, impaired perception has intestinal obstruction a lump in the abdomen Bleeding in the gastrointestinal tract, hernia, received radiation to the abdomen. Had a cesarean section within 6 months. 5) Volunteers are welcome to participate in research projects. Participants were excluded 1) after abdominal surgery within the first 6 months, 2) those who are pregnant during the research, 3) people who can't be tracked or missing during research, 4) people with chronic enteritis 5) kidney disease patients.

Research Instrument

The primary outcome measurement was a defecation evaluation using an expanded Thai version of the Bristol stool scale including 3 parameters; defecations per week, characteristics of defecation, and sensation of complete evacuation. In addition, to understand the basic patient information, including medication experience, the demographic characteristics of subjects at the baseline were observed by using a questionnaire, assessment by specialist and qualified of EC committee.

Study Intervention

After granting informed consent, the subject was randomized into the massage group and drug group following a random assignment enclosed in envelopes. There were both groups concealed by physicians and researchers. The massage group comprised 15 subjects who received a 60-minute Court Type Thai Traditional Massage. Therapy was conducted by the same registered Thai traditional medicine practitioner with over 5 years of experience throughout the study. The court type that traditional massage carried out on the subjects was called "Darn-Lom". The message according to the formula prescribed in the applied Thai traditional medicine treatment for Darn-Lom patients consists of 7 steps as follows: Step 1: Leg massage 4 lines and then press on wind gate point for 45 seconds on each side. The massage both sides. Step 2: Back

massage both sides, then press signals point 1, 2, and 3 with focus pressing on 3 signal point press for 30 seconds. Step 3: Outer leg side the leg massage on lateral both sides, then press signals point 1, 2, and 3 with focus pressing on 2 signal point press for 30 seconds. Step 4: Inner leg side the leg massage on internal both sides, then press signals point 1 and 2 with focus pressing on 1 signal point press for 30 seconds. Step 5: Abdominal massage has 2 positions consisting of the Wak position and the Narb position. The Wak position starts pressing from the lateral iliac crest on another side to the midline 3 times and the Narb position Starts from the midline pressing to the iliac crest 3 times. Step 6: Abdominal massage or was called Kouy Tong, massage around the abdomen in a circular motion with 8 points, clockwise rotation massage. Step 7: Abdominal massage on each side by focusing on signal points 1, 2, 3, and 4 lateral sides with focus pressing on 5 signal points for 30 seconds (Abdominal wind gate point), then repeatedly press for the second time on the 5 signal press points while continuously monitoring the pulse beat. The drug group comprised 15 subjects who received *Cassia alata* drug and used 1 sachet of the drug to dissolve 1 glass of warm water 7 days in a row (before bedtime). Prior to the commencement of the experiment, subjects in both groups were asked questions regarding their defecations based on a defecation evaluation form. One day after the completion of the experiment, they were asked the same questions through telephone conversations. Volunteers could terminate the intervention whenever they desired without reason and this did not affect their normal treatment.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences Version 20 (SPSS V.20). Descriptive statistics used included percentage, mean and standard deviation, while the inferential statistic used was the t-test for between-group comparisons before and after the experiment.

Results

Most of the people with chronic constipation in the Court-type Traditional Thai massage were female 60.00% and the *Cassia alata* drug group were male 80%, most of the Court-type Traditional Thai massage and the *Cassia alata* drug group had an average age between 21-25 years, most of the Court-type Traditional Thai massage group and *Cassia alata* drug group were single (100.0 and 100.0 percent), both with a bachelor's degree (90.0% and 53.3%) with constipation chronic for an average of 1 to 3 years (83.3% and 43.3%), most of them had meat diet behavior (100.0 and 90.0%), did some inconsistent exercise (70.0% and 80.0%), had flatulence and indigestion, occasional wind in the stomach (90.0 and 83.3 percent).

Within-Group Comparisons of the number and Characteristics of Defecations and Sensation of

Complete Evacuation Before and After the Experiment. Both the massage and drug groups showed improvements in a number of defecations. The number of subjects in the massage group who reported fewer than 3 defecations per week reduced. Defecations characteristics such as the number of defecations, characteristics of defecations, and sensation of complete evacuation, before and after the experiment are shown in Table 1. Dramatically from 7 to only 2. Similarly, the number of subjects in the drug group who defecated less than 3 times per week, dropped drastically from 9 before

to 1 after the experiment. The number of subjects in both the massage group and the drug group whose defecations fell into categories 1 and 2 or unhealthy on the Bristol stool scale reduced substantially to 2 for the former and zero for the latter; however, only subjects in the drug group experienced mushy, watery, or liquid defecations after the experiment. A similar pattern was found for the sensation of complete evacuation with the number of subjects falling drastically from 9 for both groups to only 3 for the massage group and from 9 for both groups to only 1 for the drug group (Table 1)

Table 1 Within-Group Comparisons of the Number and Characteristics of Defecations and Sensation of Complete Evacuation Before and After the Experiment

Questions relating to defecations	Pre-test		Post-test	
	Massage N=15	Drug N=15	Massage N=15	Drug N=15
Number of defecations per week				
Fewer than 3 times	7 (46.7%)	9 (60.0%)	2 (13.3%)	1 (6.7%)
Equal to or more than 3 times	8 (53.3%)	6 (40.0%)	13 (86.7%)	14 (93.3%)
Characteristics of defecations				
1. Separate hard lumps, like nuts (hard to pass)	1 (6.7%)	2 (13.3%)	-	
2. Sausage-shaped but lumpy	8 (53.3%)	10 (66.7%)	2 (13.3%)	
3. Like a sausage but with cracks on the surface	6 (40.0%)	3 (20.0%)	7 (46.7%)	5 (33.3%)
4. Like a sausage or snake, smooth and soft	-		4 (26.7%)	4 (26.7%)
5. Soft blobs with clear-cut edges (passed easily)	-		2 (13.3%)	2 (13.3%)
6. Fluffy pieces with ragged edges, a mushy stool	-		-	2 (13.3%)
7. Watery, no solid pieces; entirely liquid	-		-	2 (13.3%)
The sensation of complete evacuation				
Less than 25% of the total number of defecations	9 (60.0%)	9 (60.0%)	3 (20.0%)	1 (6.7%)
Equal to or more than 25% of the total number of defecations	6 (40.0%)	6 (40.0%)	12 (80.0%)	14 (93.3%)

Between-Group Comparisons of the number of characteristics of defecations and sensation of complete evacuation before and after the experiment. Results demonstrated no statistically significant differences between the 2 groups before and after the experiment in

almost all aspects. The only exception was the sensation of complete evacuation after the experiment, where the massage group differed significantly from the drug group ($P < 0.05$) (Table 2).

Table 2 Between-Group Comparisons of Frequency and Characteristics of Defecations and Sensation of Complete Evacuation Before and After the Experiment

Questions relating to defecations	Massage		Drug		p-value
	Mean	S.D.	Mean	S.D.	
Number of defecations per week					
Before the experiment	1.53	0.51	1.40	0.50	0.481
After the experiment	1.86	0.35	1.93	0.25	0.559
Characteristics of defecation					
Before the experiment	2.33	0.61	2.06	0.59	0.238
After the experiment	3.40	0.91	4.46	1.45	0.001*
The sensation of complete evacuation					
Before the experiment	1.40	0.50	1.40	0.50	1.000
After the experiment	1.80	0.41	1.93	0.25	0.299

* Significant level at 0.05

Discussion

This study aimed to the comparative study of the effectiveness of a group of Court-type Traditional Thai massage and *Cassia alata* drugs on the treatment of

chronic constipation. The results showed that both Court-type Traditional Thai massage and *Cassia alata* drug were effective in treating chronic constipation not statistically different, However, when considering on a

case-by-case basis, it was found that Court-type Traditional Thai massage can help increase the frequency of defecation and reduce the feeling of defecation in the end. But the nature of defecation differs in the types of stools based on the size of Bristol stools. Royal Thai massage and abdominal massage resulted in increased intestinal motility [5]. This increased the number of defecations per week by hundreds, 90 each, and has the appearance of a small lump, with no watery excretion while receiving *Cassia alata* drug. It was found that it could increase defecation frequency and reduce the feeling of incomplete defecation, but the defecation characteristics differed in stool type based on the size of the stools Bristol had excreted. The lump is liquid and watery, and both have side effects causing abdominal pain due to compression of the colon.

Conclusion

Both the Court-type Traditional Thai massage and the *Cassia alata* drug are effective in treating constipation. There is a difference in the appearance of the stool that outcomes. And *Cassia alata* drug has a side effect that causes abdominal pain. However, massage can be dangerous if the massage therapist is not properly trained. Therefore, treatment should only be done by a registered and licensed practitioner, such as a Thai traditional practitioner and Applied Thai Traditional Medicine.

Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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A Systematic Review of Randomized Controlled Trial on Efficacy of *Centella asiatica* for Wound Healing

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ABSTRACT

Introduction: The delay of wound healing leads to the risk of infection, chronic wound and other complications. Proper treatments for wound care will speed up the healing process and reduce scar formation. *Centella asiatica* is the famous medicinal plant among herbal skin care for healing both normal and chronic wounds with less adverse effects.

Objective: This systematic review was performed to evaluate the wound healing properties of *C. asiatica* in clinical trials.

Methods: A pre-registered systematic review was performed following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search strategy and three electronic databases including PubMed, ScienceDirect and Google Scholar were used in this study. We had intended to perform a meta-analysis, but this was not possible due to variation in study design and reporting. The search strategy identified 1,410 articles through selected databases and 5 clinical trials met the inclusion criteria for quantitative synthesis. The quality assessment for risk of bias of the selected studies was evaluated by Cochrane risk of bias tool. The inclusion criteria were randomized controlled trials. We included participants with wounds regardless of age, gender, and ethnicity. The different types of wound are burn wound, diabetic foot ulcer, hypertrophic scar, split-thickness skin graft, and post laser resurfacing.

Results: The favorable effect of *C. asiatica* was observed with a quicker wound healing, possibly due to the synthesis of collagen type 1, 2, and 3, stimulation of fibronectin, and remodeling of the extracellular matrix. Furthermore, *C. asiatica* has a beneficial effect on anti-inflammation and increases tensile strength, accelerating the formation of epithelium and keratin.

Conclusion: *C. asiatica* may have beneficial effects on wound healing; however, more clinical trials are needed in order to perform a meta-analysis to ensure the wound healing effects of the plant in different parameters.

Keywords: *Centella asiatica*; wound healing; clinical application

Introduction

Centella asiatica is a tropical herbaceous perennial plant in the Apiaceae family. The plant can be found abundantly in many tropical and subtropical countries worldwide such as Thailand, China, India and Sri Lanka [1]. *C. asiatica* is a common culinary vegetable with high nutrition and it has been traditionally used as a

medicinal herb to treat skin problems as well as to heal wounds [2]. In Thai traditional medicine, the plant is called Bao-Bok which has a cold property with a bitter taste, thus it is recommended to treat the diseases with hot symptoms e.g. inflammation. Additionally, the whole plant has been used to dispel heat, accelerate wound healing, reduce keloid formation, treat burns,

bruises, mouth ulcers as well as relieve heartburn and thirst [3]. Many studies reported that *C. asiatica* have positively promoted wound healing by improving collagen synthesis [4, 5] and microcirculatory function [6, 7]. However, most of the studies have been done in animal and in vitro models.

Wound healing is a natural process occurred in human body. There are four main phases of cellular and bio-physiological events for normal wound healing steps including hemostasis, inflammation, proliferation and remodeling [8]. In addition, there are two main factors that can delay wound healing, including: factors local to the wound itself (desiccation, infection or abnormal bacterial presence, maceration, necrosis, pressure, trauma, and edema) and systemic factors (age, body type, chronic disease, immunosuppression, nutritional status, radiation therapy, and vascular insufficiencies) [9]. The aforementioned factors impaired the healing process of wound which can lead to the pathologic inflammation of the wounds [10].

Numerous studies of *C. Asiatica* were investigated with various types of wounds and scar such as burns [11], diabetic foot ulcer [12], laser resurfacing wounds [13], split-thickness skin graft wounds [14], acne scars [15] hypertrophic scars and keloids [16].

From the aforementioned studies, there are various types of wounds treated with *C. asiatica* in clinical trial settings. All of them revealed the effectiveness of *C. asiatica* as the treatment for various kinds of wounds; however, the data have been heterogeneous on the outcome and in which type of wounds this plant should be employed. This systematic review evaluated the current evidence on *C. asiatica* in clinical trial settings (randomized controlled trial) for wound healing which is needed to provide an evidence base for researchers and practitioners. The evidence is assessed to guide clinical decision making and to further facilitate future research in a timely manner.

Methodology

This systematic review was reported according to the 2015 PRISMA recommendation [17]. The protocol of this study was previously registered with the Prospero registration no. CRD42021284821.

Search strategy

The literature search was performed by 3 authors, searching the following electronic bibliographic English databases: PubMed, ScienceDirect, Google Scholar. All databases were searched from the available date of inception through 4 September 2021. The following keywords were used: centella AND wound AND random AND clinic, centella AND wound healing AND random AND clinic, Centella asiatica AND wound AND random AND clinic, Centella asiatica AND wound healing AND random AND clinic, Gotu kola

AND wound AND clinical AND random, Gotu kola AND wound healing AND clinical AND random. Three authors independently screened the titles and abstracts for eligibility. All searches were re-conducted before the completion of this review, to retrieve any further includable studies.

Selection process

Inclusion criteria: the studies were eligible if they were randomized controlled trials (RCTs) that included *C. asiatica* for wound healing. We included participants with wounds regardless of age, gender, and ethnicity. The different types of wounds are: burn wound, diabetic foot ulcer, hypertrophic scar, split-thickness skin graft (STSG), acne and post laser resurfacing. Exclusion criteria: the studies were those not conducted on humans; those not including supplementation or topical treatment with *C. asiatica* or any of its extracts; and those involving participants under 18 years of age. The reason for excluding studies with participants younger than 18 years is due to the metabolic stress that is present during growth. Studies in another language other than English were also excluded from the review. No publication date restrictions were applied (Figure 1).

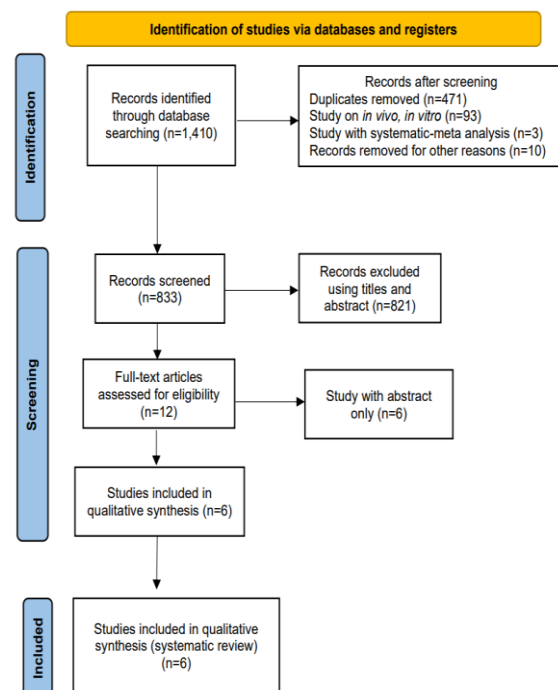


Figure 1. Flow diagram of the search strategy

Intervention groups

Use a topical ointment containing *C. asiatica* or herbal extracts containing mainly *C. asiatica* were included with the duration of treatment for at least 2 weeks.

Comparison groups

Comparison groups that received placebo or only western medications treatment were included.

Outcomes

The outcomes included in the review were: Wagner grade, skin erythema, re-epithelialization, VSS (vascularity, pigmentation, pliability, height), and VAS (visual acuity score).

Quality assessment

Three reviewers individually assessed the risk of bias of the included studies by the Cochrane risk of bias

tool (The Cochrane Collaboration, Copenhagen, Denmark) [18]. There are seven domains for evaluation including random sequence generation, allocation concealment, blinding of participants, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Three symbols including a plus (+), a question mark (?) and a minus (-) were used to indicate low risk of bias, unclear risk of bias, and high risk of bias, respectively.

Results

Five clinical randomized controlled trials [14, 15, 19-21] on wound healing utilizing *C. asiatica* were included in the current systematic review (Table 1).

Table 1 Summary of included studies

Author (Year)	Sample Size (Male/Female)	Duration	Model	Intervention	Control	Results
Yuan-Sung Kuo et al. (2012)	WH-1 cream: (4/8) hydrocolloid group: (5/7)	2 weeks	Diabetic foot ulcer	WH-1 cream containing <i>P. amboinicus</i> and <i>C. asiatica</i> in a 1: 4 ratios	Hydrocolloid fiber dressings	☑ Wagner grade improvement, ☑ Reduction in wound size (postsurgical debridement)
Amin Saeidinia et al. (2017)	CA group: (9/21) SSD Group: (10/20)	2 weeks	Burn wound	Centiderm ointment (CA)	1% Silver sulfadiazine cream (SSD)	☑ Vancouver Scar Scale (VSS) score, ☑ Visual acuity score (VAS) score, ☑ Re-epithelialization, ☑ Healing time, ☑ Infection, ☑ Pigmentation
Kamonwan Jenwitheesuk et al. (2018)	N = 23	12 weeks	split-thickness skin graft (STSG)	7% w/w Centella asiatica extract in cream preparation	Placebo	☑ Vancouver Scar Scale (VSS) score ☑ Pigmentation
Palakorn Surakunprapha et al. (2020)	Herbal extract + silicone gel: (10/14) Placebo gel: (14/10)	6 months	Hypertrophic scar	15% Herbal extract mixture (<i>Allium Cepa</i> extract, <i>Centella Asiatica</i> extract, <i>Aloe Vera</i> extract and Paper Mulberry extract) + silicone gel	Placebo	☑ Scar amelioration in height ☑ pliability ☑ vascularity ☑ pigmentation
Wilawan Damkerngsunto m et al. (2020)	0.05% w/w Eca 233 gel: (5/25) Placebo gel: (5/25)	3 months	Post laser resurfacing	0.05% w/w ECa 233 gels	Placebo	☑ Erythema, ☑ Wound appearance, ☑ Epithelialization

Risk of bias assessment

Risk of bias among five articles was summarized in figure 2. The articles in the studies were categorized as low risk of bias.

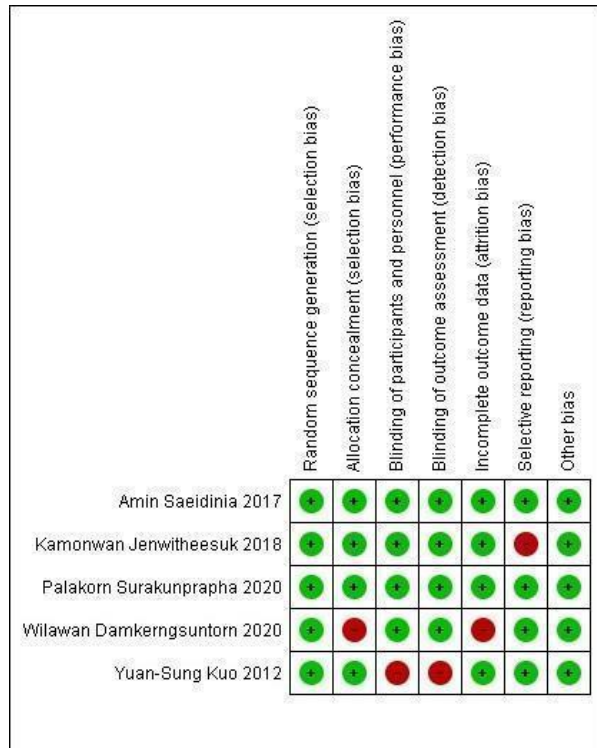


Figure 2. Risk of bias summary for the included study. Low risk of bias (+), unclear risk of bias (?), and high risk of bias (-)

Effects of interventions

The effective rate of *C. asiatica* utilization for wound healing in human from five articles were based on Wagner grade, skin erythema, re-epithelialization, VSS (vasculature, pigmentation, pliability, height) and VAS (visual acuity score).

Wagner grade evaluation

Yuan-Sung Kuo and others [19] reported a slightly reduction in Wagner grade and wound size for diabetic patients with foot ulcers. In this research, 24 patients were treated with a topical cream (WH-1) containing *P. amboinicus* and *C. asiatica* extracts or hydrocolloid fiber for 2 weeks. The improvement of Wagner grade was found in 90.9% of patients in WH-1 cream group; whereas, 70% of patients in control group had shown the improvement in Wagner grade. Wound size is one of the parameters in Wagner grade classification system, thus wound size in WH-1 cream group also reduced. However, there is no significant difference between groups for both outcomes.

Skin erythema and wound appearance

In a clinical trial research carried out by Wilawan Damkerngsuntorn and others [15], thirty participants with facial acne scars underwent a treatment with 2940 nm Er: YAG laser. Half side of the face was randomized to receive 0.05% w/w ECa 233 gel *C. asiatica* and the other side a placebo gel. The gels were applied 4 times daily for 7 days then twice daily for 3 months. The skin treated with *C. asiatica* showed statistically significant improved erythema and wound appearance at days 2, 4, and 7 (p-value < 0.05).

VSS Scores

Three trials [14, 20, 21] measured the effect of *C. asiatica* treatment on Vancouver Scar Scale (VSS). Kamonwan Jenwitheesuk and others [14] demonstrated the effect of centella cream comparing to placebo with the participants who underwent a split thickness skin graft (STSG) operation. Each intervention was applied at least 2 weeks after 14 days of epithelialization. At the end of the intervention period, only 23 patients completed the assessment. The VSS assessment was taken three times at 4, 8 and 12 weeks. It was found that pigmentation, one of the VSS parameters of experimental group at 8 and 12 weeks as well as between 4 and 12 weeks; placebo group at 12 weeks and between 4 and 12 weeks showed significant differences with p-value less than 0.05. The total VSS scores between 4 and 12 weeks of experimental group was also showed significant differences with p-value less than 0.05. However, for height in experimental group, it was worse at 4 weeks (p-value = 0.043). Amin Saeidinia and others [20] investigated the healing efficacy of Centiderm ointment, a derivative of *C. asiatica* on second-degree burn wounds when compared to silver sulfadiazine, the gold standard topical burn therapy. All VSS parameters were lower in the intervention group compared to the control group (p-value = 0.001). However, pigmentation did not reach statistical significance until day 14 of treatment (p-value = 0.001).

Palakorn Surakunprapha and others [21] utilized topical silicone gel plus 15% of herbal extract mixture (*Allium Cepa*, *Centella Asiatica*, *Aloe Vera* and *Paper Mulberry*) or placebo for 48 post-median sternotomy patients for 6 months. The scar is observed by plastic surgeons using the VSS. After 6 months all parameters in the intervention group were greater than the control group. In particular, the pliability and height were significantly greater in numbers of improvement in the intervention group than in the placebo group (p-value < 0.05). A reason for this outcome might be the effect of either silicone gel and herbal extract. Silicone gel is able to simulate the homeostasis of the stratum corneum and can also facilitate regulation of fibroblast production and reduction in collagen production. These effects help to soften the scar leading to scar reduction. Additionally,

the formation of scar can be reduced by inhibitory effect of *C. asiatica* on the expression of the transforming growth factor-beta (TGF- β) 1 and 2.

VAS Scores

The visual acuity score (VAS) was evaluated in the study of Amin Saeidinia and others [20] on burn wound. The declination of VAS score in day 3,7,14 in CA group exhibited better healing than that of the control group (SSD group) with p-value less than 0.001.

Re-epithelialization

In a clinical trial carried out by Amin Saeidinia and others [20] treated 75 patients with second-degree burn wounds on their limbs. These patients were administered topically treated once a day, with either Centriderm cream (a topical ointment containing *C. asiatica*) or 1% silver sulfadiazine cream. Re-epithelialization in the intervention group was 13.7 ± 1.48 days compared to 20.67 ± 2.02 days in the control group (p-value < 0.001). For this outcome might be the effect of *C. asiatica* on the production of vascular endothelial growth factor (VEGF) resulting by the increase in monocyte chemoattractant protein-1 (MCP-1) expression in keratinocytes and the increase in IL-1 β expression in macrophages leading to wound healing acceleration.

Discussion

Wound healing effect from *C. asiatica* was mentioned in traditional medicine in many countries such as India, Sri Lanka, China and Thailand [22]. The current systemic review of *C. asiatica* for wound healing found that the herb may provide a positive effect on wound healing. The phytochemical constituents in *C. asiatica* have been used to support the claim for its therapeutic effect. Previous clinical study of ECa 233 gel composing of madecassoside 51% and asiaticoside 38% exhibited the improvement of erythema and wound appearance [15]. Asiaticoside, a triterpene constituent in *C. asiatica* has been studied for wound healing activity in normal and diabetic animal model [23]. The compound has been reported to improve wound healing process through collagen formation and angiogenesis; improve the tensile strength of the newly skin formation; inhibit the inflammatory process; improve the capillary permeability [7, 24]. Antioxidants play an important role on wound healing [25]. In animal model, asiaticoside obtained from *C. asiatica* possessed the ability to enhance antioxidant level in tissue leading to the enhancement of wound healing [26]. Previous study of asiaticoside obtained from *C. asiatica* treated with normal and keloid fibroblasts have revealed the ability of the compound to decreased fibroblast cell proliferation in a time- and dose-dependent manners; inhibit mRNA expression as well as type 1 and type 3 collagen protein expression; decrease TGF- β RI and

TGF- β RII expression; promote the expression of Smad7 protein and mRNA [27]. The increment of collagen formation may be due to vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [28]. During angiogenesis VEGF increases the vascular permeability. The mechanism by which VEGF is stimulated results from increased expression of IL-1 β and Monocyte Chemoattractant Protein-1 (MCP-1) [28]. In addition, the *C. asiatica* has anti-inflammatory effects so that it can cause the reduction in IL-1 β , IL-6 and TNF α , as well as prostaglandin E2 (PGE2) [29]. IL-1 β , IL-6 and TNF α are pro-inflammatory cytokines secreted by inflammatory cells. These substances can stimulate cyclooxygenase enzymes that convert arachidonic acid to prostaglandin, especially prostaglandin E2 [30]. PGE2 plays a role in the regulation of the immune response and blood pressure. As a result, during inflammation, PGE2 can cause swelling, redness, and pain around the wound [23]. Therefore, *C. asiatica* may decrease these substances by reducing inflammation in the affected area [31]. The current study had several limitations. The number of included studies was only 5 studies in the systematic review, were tested, varied between the studies, making comparisons between them exceedingly difficult. Therefore, it was not possible to conduct a meta-analysis to assess the effect of *C. asiatica* on the reviewed outcomes. Thereby further clinical studies are needed to evaluate the effectiveness of *C. asiatica* in wound healing.

Conclusion

Based on the five articles included in this systematic review, the therapeutic potential of *C. asiatica* in terms of its efficacy and versatility for wound healing might show the good outcome for this property. The most prominent mechanism is to increase collagen synthesis due to increased angiogenesis and its anti-inflammatory effect. Furthermore, this anti-inflammatory effect may result in reduced swelling, redness, and pain in the wound area due to the lessening of prostaglandin E2 (PGE2) and other inflammatory factors.

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Effect of a Pain Management Combined with Mahajak Oil Medicine Program in Elderly with Osteoarthritis: A Case Study of Thay Nam Health Promoting Hospital, Pho Thale District, Phichit Povince

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ABSTRACT

Introduction: Osteoarthritis (OA) is a chronic, degenerative, musculoskeletal disease with global prevalence of 20% of women and 10% of men over 60 years of age. Most patients with OA of the knee will develop progressive functional limitation and physical disability with age. The prevalence of symptomatic OA of the knee among elderly Thai people was reported as 10.40% (women) and 4.20% (men). The disabling symptoms of knee OA in older patients, such as chronic pain and joint stiffness potential to cause psychological changes in patients, leading to the quality of life of older

Objective: were to study results of pain management Combined with Mahajak oil medicine program and compare knowledge, attitude, Levels of knee pain and Degree of passive knee flexion on Elderly

Methods: quasi-experimental research with three-groups and repeated-measures design. The sample group was selected with purposive sampling. The 90 cases of elderly were patients with the severity of the disease osteoarthritis Mild to Severe (39-20 points). They were separated into three groups into 3 groups, each group of 30 cases. The experimental group received pain management program Combined with Mahajak oil medicine. The control group received the program together with warm water and the control group without any treatment. One-way repeated measures ANOVA was employed for comparing knowledge scores, attitudes, Levels of knee pain and Degree of passive knee flexion among the experimental group and two control groups.

Results: The findings indicated that the experimental group with Mahajak oil medicine usage has average score of knowledge, attitude, and Levels of knee pain higher than pretest and attitude and Levels of knee pain higher than the results of control groups with statistical significance (p -value<0.05). Besides, the results of experimental group with Mahajak oil medicine usage proved that the average range of Degree of passive knee flexion were better than before the program after the fourth week and twelfth week. The results of the fourth week and twelfth week were statistically significant difference with the first week (p -value<0.05). For pair comparison, it was found that there were two different pairs: the group with Mahajak oil medicine usage and the control group. Meanwhile, no statistically significant difference was found between the group with warm water and the control group (p -value<0.05).

Conclusion: pain management program Combined with Mahajak oil to provide effective health services

Keywords: *pain management; elderly; osteoarthritis*

Introduction

The elderly population is increasing and degenerative-related diseases in the elderly are also becoming more common. This includes 80-90% of cases

of osteoarthritis in people over 65 and more in women than men over age 55. It causes knee pain while moving. Knee stiffness and reduced range of motion affect the daily work activities. including the mental state and

quality of life of the patients [1,2]. Thailand tends to have an aging population continuously increasing, causing the country to step into an aging society. Old age is the age of deterioration of the body and various organs. Osteoarthritis of the knee is one of the causes of disability and affects health conditions in the elderly [3]. Thay Nam Sub-district, Pho Thale District, Phichit Province There are 1,019 elderly people out of a total population of 5,816, representing 17.46 percent, who have entered an aging society or will become a "full aged society" (Aged Society) when the proportion of the population is 60 years old. up to 20 percent [4]. Osteoarthritis, it is a condition caused by the deterioration of joint cartilage. This causes cartilage to be unable to support weight and loses the properties of the knee fluid. When the knee is moved, friction occurs, and wear and tear of cartilage occurs. The surface of the cartilage will Hard, uneven surface causing pain when moving or putting on weight [1,5]. Pain in elderly was often unrecognized and undertreated. Ineffective pain management can have a significant impact on the quality of life of older adults, leading to depression, social isolation, and a loss of function. Proper assessment of elderly requires the physician to regularly ask about the presence of pain and be skillful in assessment strategies to evaluate the frequency and intensity of pain. Assessment of pain in elderly with dementia and communication disorders is especially challenging. Effective pain management in elderly patients should include both pharmacologic and nonpharmacologic strategies. Polypharmacy, drug-drug and drug-disease interactions, age-associated changes in drug metabolism, and the high frequency of adverse drug reactions need to be carefully considered in using medications in this population. Nonpharmacologic approaches such as cognitive-behavioral therapy, education, osteopathic manipulative treatment, and exercise should be applied in addition to pharmacologic therapy. Using a team approach and incorporating principles of pain management [6,7]. There are several treatment approaches, including weight control. change in daily behavior Avoid excessive bending of the knees, physical therapy, muscle training. Knee bandages may be used. knee splint but if used for a long time will cause muscle atrophy. Taking medications to relieve symptoms, such as pain relievers and anti-inflammatory drugs. muscle relaxant but if used continuously for a long time. Will cause side effects such as stomach ulcers, kidney failure, high blood pressure, etc [8-10]. Treatment of osteoarthritis of the knee with Thai traditional medicine There are both oral and external medicinal types, such as knee wraps, oil massages, etc., which are herbal compresses. It is a herbal remedy with essential oils and the heat of the compress to help improve blood circulation. The compress is also used in conjunction with massage to help stimulate better blood circulation. reduces swelling Relieve inflammation of

the muscles around the knee [11,12]. Mahajak Oil Medicine was a Thai traditional medicine that are selected from many herbs with extraction of oil So that elderly people with knee pain from osteoarthritis can be used to relieve initial knee pain on their own. Prevent or correct any deformed clauses. which properties from the specific properties of each type of drug Thus, it was assumed that Mahajak oil might be effective in reducing pain due to its anti-inflammatory effect. and can also heal wounds from antibacterial activity anti-inflammatory effect accelerate wound healing [13,14,15]. The research team has studied Mahajak oil. Used to solve the problem of knee pain from osteoarthritis in the elderly by adding herbal medicines. Which has properties to reduce inflammation, relieve pain, swelling, tight lines, aches and pains to be used as an ingredient in the Mahajak Sutra oil recipe of Thay Nam Sub-District Health Promoting Hospital, Pho Thale District, Phichit Province with the oil containment procedure So that elderly people with knee pain from osteoarthritis can be used to relieve initial knee pain on their own. Prevent or correct any deformed clauses. for the elderly to have a better quality of life It can be used as a guideline for treating and alleviating the symptoms of osteoarthritis for the elderly and the general public in Tai Nam Sub-District Health Promoting Hospital and can be disseminated to hospitals or organizations. other agencies.

Objective

To study results of pain management program Combined with Mahajak oil medicine on Elderly in Thay Nam Health Promoting Hospital.

Sub-Objectives

1. To study the comparison of pain management knowledge, attitude, Levels of knee pain and Degree of passive knee flexion within experimental group
2. To study the comparison of Levels of knee pain and Degree of passive knee flexion between the experimental group and the control groups.

Hypotheses

1. After implementing the intervention, the experimental group, receiving the pain management program Combined with Mahajak oil medicine, had a higher mean score of pain management knowledge, attitude, Levels of knee pain and Degree of passive knee flexion than before providing the program.
2. After implementing the intervention, the experimental group, receiving the pain management program Combined with Mahajak oil medicine, had a higher mean score of pain management knowledge, attitude, Levels of knee pain and Degree of passive knee flexion than the control groups.

Methodology

This research is a three-group pre- and post-test quasi-experimental design. The experimental group received the pain management program Combined with Mahajak oil medicine, while the first control group received the pain management program with massage using warm water and the latter control group received none of these interventions. The current research studied the pain management program combined with Mahajak oil medicine in by comparing the levels of pain management knowledge, attitude, Levels of knee pain and degree of passive knee flexion before and after participating in the experiment.

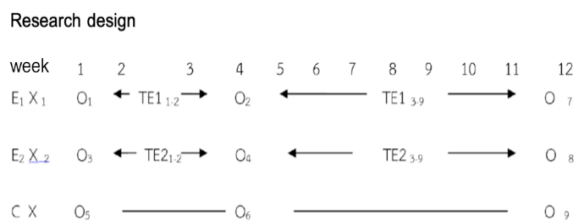


Figure 1 Research design

Figure 1 E1 means the experimental group who received the pain management program Combined with Mahajak oil medicine, E2 means the control group who received the pain management program with massage using warm water, C mean the control group who received none of the interventions, O means levels of pain management knowledge, attitude, Levels of knee pain and degree of passive knee flexion, X means a standard of care pain management program, X1 means the pain management program Combined with Mahajak oil medicine, X2 means the pain management program with massage using warm water, TE11-9 means phone call follow-ups to encourage participation at week 2,3,5,6,7,8,9,10, and 11 to spot on problems and obstacles as well as to appreciate the experimental group to practice the interventions continuously and regularly, and TE21-9 means phone call follow-up to encourage participation at week 2,3,5,6,7,8,9,10, and 11 to the control group receiving pain management program with massage using warm water with to practice the interventions continuously and regularly.

The sample group of this study was elderly in Thay Nam Health Promoting Hospital. The sample group was selected with purposive sampling. The 90 cases of elderly were patients with the severity of the disease osteoarthritis Mild to Severe (0-39 points). Between March – June 2021. The samples were selected using a purposive sampling method according to the following criteria: elderly ages of over 60 years who had fully conscious and were able to communicate. One hundred five people met the criteria and were included in the study. These number was equally divided into three groups, including one experimental group (35 people) and two control groups (35 people each) (Figure 2). Fifteen participants disappeared from the study due to changing to new health service, visiting relatives or grandchildren in other provinces, being admitted at hospital, and being paralysis. A total of 90 participants remained, including 30 people in the pain management program Combined with Mahajak oil medicine, 30 people in the first control group attending the pain management program with massage using warm water, and 30 people in the other control group receiving none of these interventions.

Research instruments

Data-collection instruments included an assessment tool for knowledge, attitude, Levels of knee pain and Degree of passive knee flexion and a questionnaire. The questionnaire passed the content validity by three qualified individuals. The content validity was calculated using Rovinelli and Hambleton’s formula with the IOC between 0.94 - 1.00.

This questionnaire was modified before the implementation by testing with 30 elderlies around Bueng Na Rang Health Promoting Hospital. The reliability calculated by KR-20 was 0.73. The Cronbach's alpha coefficient of the questionnaire assessed knowledge, attitude, Levels of knee pain and Degree of passive knee flexion was 0.85 and 0.89, respectively.

The experimental tool consisted of a pain management program Combined with Mahajak oil medicine who felt numb, lesson plan, videos.

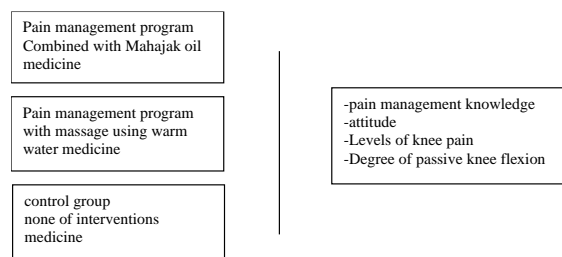


Figure 2 Cenceptual Framework

Results

The comparison of the mean score of knowledge, attitude, levels of knee pain and degree of passive knee flexion between the experimental and the control groups before and after the experiment were shown in table 1. After the 12-week experiment, the mean of knowledge, attitude, levels of knee pain and degree of passive knee flexion of the experimental group, receiving the pain management program Combined with Mahajak oil medicine, was significantly higher than pretest at week 1 and post-test at week 12 (p-value<0.05).

The results from the pairwise comparison indicated that were shown in table 2. There was a significant difference in the mean of pain management knowledge

in the experimental group between pre-test week 1 and post-test at week 4 and 12 (p-value<0.05), and there was no difference between post-test week 4 and post-test week 12.

The results of the pairwise comparison levels of knee pain there was a significant difference between the experimental group (Mahajak oil use), the control group (warm water use) and the control group (non-intervention) at post-test week 12 (p-value<0.05), and there was no difference between control group (warm water use) and the control group (non-intervention) were shown in table 3.

Table 1 The comparison of the mean knowledge, attitude, levels of knee pain and degree of passive knee flexion before and after receiving the pain management program combined with Mahajak oil medicine (the experimental group) using One-Way Repeated Measures ANOVA (n=30)

Variables	The experimental group (n=30)				
	\bar{x}	SD	df	F	p-value
Pain management knowledge					
Pre- test week 1	3.90	2.19	29	17.88	< 0.01*
Post-test week 4	5.93	1.20			
Post-test week 12	6.03	1.27			
Attitudes					
Pre- test week 1	12.17	1.70			
Post-test week 4	14.70	2.83	29	62.645	< 0.01*
Post-test week 12	17.53	2.06			
Knee pain					
Pre- test week 1	25.73	2.88			
Post-test week 4	29.90	2.34	29	20.45	< 0.01*
Post-test week 12	31.43				
Degree of passive knee flexion					
Pre- test week 1	2.00	0.00			
Post-test week 4	3.03	0.12	29	70.90	< 0.01*
Post-test week 12	3.47	0.16			

Table 2 The pairwise comparison of the mean of pain management knowledge of the elderly before and after receiving the pain management program combined with Mahajak oil medicine (the experimental group) (n = 30)

Pairwise comparison	Mean different	p-value
Pre-test week 1 – post-test week 4	-2.53	< 0.01*
Pre-test week 1 – post-test week 12	-5.37	< 0.01*
Pre-test week 4 – post-test week 12	-1.83	< 0.06

Table 3 Multiple comparisons of Levels of knee pain classified by groups using Tamhane’s T2

Group i	Group j	Mean Difference (i-j)	p-value
Experimental group (herb use) (\bar{x} = 31.43)	Control group (warm water use) (\bar{x} = 27.80)	3.63	<0.01*
Experimental group (herb use) (\bar{x} = 31.43)	Control group (non-intervention) (\bar{x} = 25.50)	5.93	<0.01*
Control group (warm water use) (\bar{x} = 27.80)	Control group (non-intervention) (\bar{x} = 25.50)	2.30	0.09

The results of the test for difference of the overall mean of degree of passive knee flexion revealed that there was a significant difference in the overall mean degree of passive knee flexion among the experimental group, the control group, and the other control group (receiving non-intervention) (p-value<0.05) that were shown in table 4.

Table 4 Comparison of Degree of passive knee flexion among the experimental, the control, and the other control group at 12- week post-experiment using One-Way ANOVA

Groups	n	\bar{x}_t	Numbness		
			F	df1/df2	p-value
Experimental group	30	8.50	77.57	2/86	<0.01*
Control group (warm water use)	31	6.10			
Control group (non-intervention)	28	6.00			

*Level of numbness \bar{x}_t (sum means)

The results of the multiple comparison test, classified by groups indicated that there was a significant difference in the degree of passive knee flexion between the experimental group and the control group, and between the experimental group and the control group (receiving non-intervention) (p-value<0.05). In contrast, there was no difference between the control group and the other control group (receiving non-intervention).

Table 5 Multiple comparisons of Degree of passive knee flexion classified by groups using Tamhane's T2 method

Group i	Mean Group j	Mean Difference (i-j)	p-value
Intervention group ($\bar{x}_i = 8.50$)	Control group ($\bar{x}_j = 6.10$)	2.40	0.01*
Intervention group ($\bar{x}_i = 8.50$)	Control group (non-treatment) ($\bar{x}_j = 6.00$)	2.50	<0.01*
Control group ($\bar{x}_i = 6.10$)	Control group (non-treatment) ($\bar{x}_j = 6.00$)	0.10	0.46

*Degree of passive knee flexion \bar{x}_i (sum means)

Discussion

According to the results of the study, the researcher analyzed and discussed as the following hypotheses.

1. The comparison of the mean score of knowledge, attitude, Levels of knee pain and Degree of passive knee flexion between the experimental and the control groups before and after the experiment were shown in table 1. After the 12-week experiment, the mean of knowledge, attitude, Levels of knee pain and Degree of passive knee flexion of the experimental group, receiving the pain management program Combined with Mahajak oil medicine, was significantly higher than pretest at week 1 and post-test at week 12 (p-value<0.05).

2. There was a significant improvement in knowledge, attitude, Levels of knee pain and Degree of passive knee flexion of the experimental group (receiving the pain management program Combined with Mahajak oil medicine), the control group (receiving the pain management program with massage using warm water) and Control group (non-treatment) was significantly higher than pretest at week 1 and post-test at week 12 (p-value<0.05).
3. After completing the experiment, the mean score of knowledge, attitude, Levels of knee pain and Degree of passive knee flexion of the experimental group (receiving the pain management program Combined with Mahajak oil medicine) and the control group (receiving the pain management program with massage using warm water) was significantly better than that in the control group (receiving non-intervention) (p-value<0.05).
4. Pain management knowledge: knowledge among the experimental group and the control group (receiving the pain management program with massage using warm water) increased. This improvement is likely to be impacted by the pain management program applied the Bloom's taxonomy. This theory proposes that successful teaching and learning results from the preparation of the instructor and for teaching materials. In this study, the instructors stimulated learning by appropriate lectures and group discussion and employed various resources for the intervention, consisting of videos, a pain management handbook, a Mahajak oil medicine handbook, and role models. Moreover, the learners gained knowledge from the information received, analyzed, synthesized, and combined old and new knowledge as a result in improvement in the knowledge of the experimental group and the control group receiving the program. This aspect is consistent with Sansila et al [16], who studied the outcomes of pain management program, adopted the Bloom's taxonomy (1975) and the Subcharoen [17], for family caregivers with elderly in the area of responsibility of Srithep, Phetchabun Province. This study suggested that the intervention group showed a significantly higher mean score in knowledge, attitude, than the control group (p-value<0.05) Sansila et al [16]. Moreover, this result is in line with Cavalieri [18], who studied the effect of a foot care program on foot care knowledge and behavior of older patients with type 2 diabetes in Roi-Et Hospital and found that after completing the intervention, the mean knowledge and behavior score in the test group was higher than that at baseline and of the control group (p-value<0.05). This positive result is likely to result from the participatory learning by a group process, which allowed encouraging the participants to solved problems, exchanged experiences and improved

their skills. This aspect is also associated with Bruckenthal [19], who suggested that providing foot care-related information, knowledge, demonstrations, and practices brings about an increase in knowledge and self-care practices.

5. Attitudes towards pain management: there was a significant improvement in attitudes among the intervention (receiving the program combined with herbal foot baths) and the control group (receiving the program combined with warm foot baths) (p -value <0.05). This positive result is likely to result from the foot care program that adopted the Bloom's Taxonomy. It can be explained that when learners receive information and understand it, values, attitude, and belief will be developed. These components encourage the learners to be willing to receive any positive stimulators rather than negative ones. Consequently, they accept and realize that what they have learned is worthwhile. Besides, teaching activities should be embraced with a suitable environment and always insert what can create positive feelings to change the learners' attitudes in the desired way. This is consistent with the study of Halaszynski [20] which studied the effect of intention, attitude, and motivation on changing in weight-loss behavior and suggested that if individuals believed that actions performed display a positive effect, they are likely to have positive attitudes towards and behave such actions. This result is associated with Kuptniratsaikul et al [21] who found that after completing the experiment, the test group mean score of knowledge, attitude towards diabetes, perceived self-efficacy, outcome expectation of self-care practices, goal setting for blood sugar control practices in dietary consumption, regular exercise, stress management, hygiene, diabetic medication adherence, and reliance on doctor's appointments, was significantly higher than that before implementing the intervention and of the control group (p -value <0.05).
6. Range of motion was a significant difference in the degree of passive knee flexion between the experimental group and the control group, and between the experimental group and the control group (receiving non-intervention) (p -value <0.05). In contrast, there was no difference between the control group and the other control group (receiving non-intervention). This study has documented those herbs in Mahajak oil medicine with the effect of dispelling cold-damp, promoting blood circulation, and relieving pain, such Chiranthanut et al [22], Bruckenthal [23], Pornphol et al [14], Saenmuang et al [15] which were the major components of Mahajak oil medicine. Furthermore, results of analyzing both the herbs' frequencies and their effects were consistent. Formulae of Junlatat [11]

were mainly based on was documented in formulae by the Thai Pharmaceutical Bureau for Benevolence to relieve pain, so that the wind-cold-damp evil might be relieved. Pharmacological studies have confirmed its anti-inflammatory, analgesic, and immunosuppressive role, so it has a good therapeutic effect on the early and mid-OA. Topical use is mainly for removing wind-damp evil to warm.

Conclusion

1. For better self-care practices and better quality of life of elderly, there should be an establishment of the pain management program Combined with Mahajak oil medicine in health services.
2. Future studies should conduct long-term followup to verify whether the benefits are maintained. Another limitation was the smaller response rate to detect the desired significance level. It was not due to sample size alone which was rigorously calculated, rather, it could be explained by the fact that the cohort we recruited with relatively mild degree of knee pain at baseline might be insensitive in identifying improvement compared with high-response populations. We had attempted to analyze data by stratification of knee pain, but the result still unchanged, indicating a really no difference in this time interval. In addition, although VAS is a validated measure for pain, its estimate performed by patients with chronic pain may be imprecision [24,25]. Therefore, both objective and subjective status of knee pain assessed separately by participant and investigator could be considered in future work.

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Research on Operation Method of Tuina Treatment of Constipation in Children Based on Data Mining Technology

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ABSTRACT

Objective: To explore the Tuina manipulation (manipulation and acupoints) for treating pediatric constipation.

Methods: Randomized controlled clinical studies (RCTs) on treating pediatric constipation by Traditional Chinese Medicine (TCM) Tuina were searched from the self-established database of PubMed, Web of Science, Cochrane Library, CNQI, VIP, and Wanfang database to December 2020. SATI, UCINET, and other data mining software were used to analyze the frequency statistics, visualization centrality and support degree of relevant operation methods (manipulation + acupoint) of TCM Tuina treatment for pediatric constipation, and SPSS 26.0 were used for cluster analysis.

Results: A total of 874 pieces of literature were retrieved, and 133 RCTs literature were included after screening. Data shows 101 undifferentiated constipation prescriptions and 94 differentiated constipation prescriptions (including 48 excessive constipation and 46 deficient constipation). The results show undifferentiated constipation with abdominal rubbing manipulation (frequency 70, support 69.3%), pushing down Qijiegu manipulation (frequency 69, support 68.3%), clearing the Large Intestine manipulation (frequency 69, support 68.3%) has the highest centrality, which is a commonly used in combination. For excessive constipation, Clearing the Large Intestine manipulation (frequency 40, support 83.3%), pushing down Qijiegu manipulation (frequency 40, support 83.3%), abdominal rubbing manipulation (frequency 38, support 79.2%), Tuiliufu manipulation (frequency 35, support 72.9%) and the centrality is the highest, which is a commonly used in combination. For deficient constipation, strengthening spleen meridian (frequency 40, support 87.0%), spine pinching manipulation (frequency 37, support 80.4%), pushing Sanguan manipulation (frequency 32, support 69.6%) with the highest centrality, which is a commonly used in combination.

Conclusion: The main idea of pediatric Tuina treatment of constipation is the dredging method. They are usually treated by rubbing the abdomen, pushing down Qijiegu, and clearing the Large Intestine manipulation. Tuisanguan and clear away heat and toxic material manipulations are frequently used to treat excessive constipation. For deficient constipation, strengthening spleen meridian, spine pinching, and Tuisanguan manipulations are frequently used to replenish Qi, strengthen the spleen and relieve constipation.

Keywords: Data mining technology; Tuina; Constipation in children; Operation; manipulation; acupoint

Introduction

便秘 (constipation) 是指一种 (组) 临床症状, 表现为排便困难和/或排便次数减少 (每周排便<3次)、粪便干硬等[1]。有研究报道, 全球范围内儿童功能性便秘患病率从 0.5%到 32.2%不等,

合并患病率为 9.5%, 其中男童患病率为 8.6%, 女童为 8.9% [2]。中国儿童功能性便秘总患病率为 6.0% [3], 4~11岁、11~16岁的发病率分别为 7.47%、6.25% [4]。便秘是一种儿童常见的胃肠系

统疾病[5]，常可引起食欲减退、腹胀腹痛、睡眠不安等症，严重者还容易导致肛裂，诱发肠梗阻、发育迟缓或行为问题等[6]，严重影响儿童的生长发育和身心健康。近年来，随着人们生活饮食习惯的改变，儿童便秘的发病率呈逐年升高趋势。目前西医学对儿童便秘的发病机制尚未完全明确，临床上多以对症治疗，暂时缓解症状为主，远期效果欠佳，药物依赖性大[7]。中医小儿推拿疗法作为具有中医特色的健康疗法，以其治疗无痛苦，儿童易接受，疗效显著且无毒副作用的特点，备受医生和家长的青睐。文献显示推拿对增加儿童的排便次数，减少排便的时间具有较好的疗效[8]。在治疗上，除了依据不同证型选择不同功效的穴位外，不同的证型选择不同的手法也十分的重要[9]。本研究对小儿推拿治疗儿童便秘在操作法（手法+选穴）上的应用规律进行分析，以期能为临床治疗提供依据。

Methodology

1. 文献检索

检索收录于 PubMed、Web of science、Cochrane library、知网、维普、万方数据库（自建库至 2020 年 12 月）中医推拿治疗小儿便秘的临床随机对照研究，检索词为“便秘”、“实秘”、“虚秘”且“推拿”或“按摩”且“小儿”或“儿童”。英文检索词为（“constipation” or “excessive constipation” or “deficient constipation”） and （“tuina” or “massage”） and （“children” or “infantile”）。

2. 纳入标准

以中医推拿、按摩为主要干预手段；临床随机对照试验（randomized controlled trials, RCTs）；诊断为便秘、实秘、虚秘。

3. 排除标准

动物实验、文献综述等非临床研究文献；非中医推拿为主要干预手段的文献；推拿手法不明确的文献；重复性研究。

4. 研究方法

对推拿治疗便秘的文章进行全文阅读，检索出便秘相关手法及穴位。按照 SATI 导入格式制作 txt 录入模板，将文章标题、作者、病名、手法选穴等信息录入 txt 模板内。经 SATI 进行格式转换，并提取相关词条频次、排序等信息。然后运用社会网络分析软 UCINET 及 NetDraw 对便秘及操作法（手法+穴位）进行可视化中心度分析。并使用 SPSS 26.0 软件对使用频次≥10 的操作法（手法+穴位）进行聚

类分析，采用 Ward 连接，分析操作法（手法+穴位）的潜在组合规律。

Results

1. 文献检索结果

中文数据库共检索到 815 篇，剔除重复文献 449 篇、不符合纳入标准 233 篇，剩余 133 篇；外文数据库收集 59 篇，剔除重复文献 43 篇，不符合纳入标准 16 篇。最终纳入研究的文献 133 篇。筛选流程图总结见图 1。

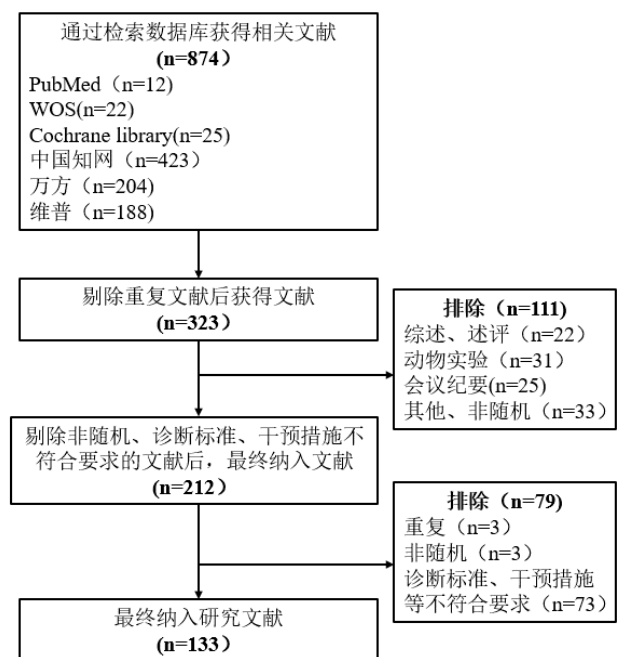


图 1 文献筛选流程图

2. 文献纳入情况

据上述关键词检索并筛选，最后纳入相关文献 133 篇（部分文献同时涉及不分型、虚秘和/或实秘），推拿操作处方不分型便秘 101 个，分型便秘 94 个（包括实秘 48 个、虚秘 46 个），操作法（手法+穴位）总频数 1457 次。

3. 推拿操作法

（手法+穴位）频次、支持度分析如图 2、表 1 所示，在不分型便秘中摩腹、推下七节骨、清大肠三者的使用频次较高，支持度>50%，其中摩腹支持度最高（69.3%），应用最多。实秘中清大肠、推下七节骨、摩腹、退六腑的使用频次较高，支持度>70%，其中清大肠支持度最高（83.3%），应用最多。虚秘中补脾经、捏脊、清大肠、推三关、摩腹、揉足三里的使用频次较高，支持度>50%，其中补脾经支持度最高（87.0%），应用最多。

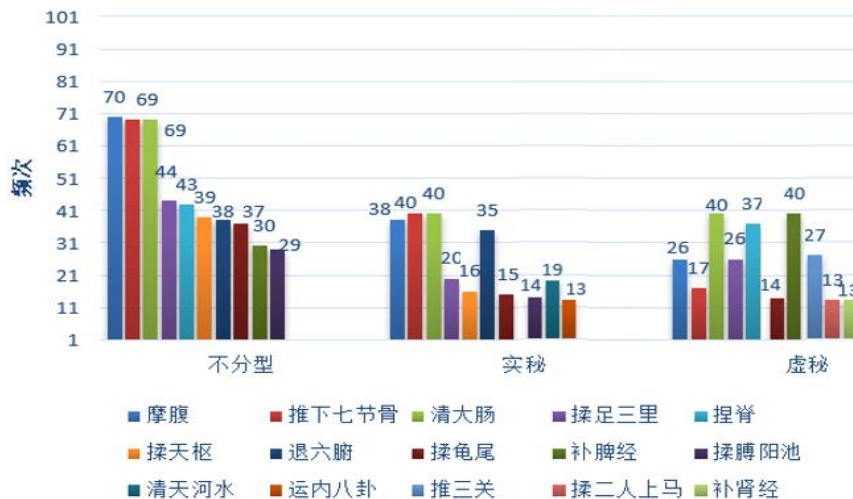


图 2 推拿操作法（手法+穴位）前 10 项

表 1 推拿操作法(手法+选穴)频次、支持度

类型	操作法	频次	支持度 (%)	操作法	频次	支持度 (%)
不分型便秘	摩腹	70	69.3%	揉天枢	39	38.6%
	推下七节骨	69	68.3%	退六腑	38	37.6%
	清大肠	69	68.3%	揉龟尾	37	36.6%
	揉足三里	44	43.6%	补脾经	30	29.7%
	捏脊	43	42.6%	揉膊阳池	29	28.7%
实秘	清大肠	40	83.3%	清天河水	19	39.6%
	推下七节骨	40	83.3%	揉天枢	16	33.3%
	摩腹	38	79.2%	揉龟尾	15	31.3%
	退六腑	35	72.9%	揉膊阳池	14	29.2%
	揉足三里	20	41.7%	运内八卦	13	27.1%
虚秘	补脾经	40	87.0%	揉足三里	26	56.5%
	捏脊	37	80.4%	推下七节骨	17	37.0%
	清大肠	32	69.6%	揉龟尾	14	30.4%
	推三关	27	58.7%	揉二人上马	13	28.3%
	摩腹	26	56.5%	揉膊阳池	13	28.3%

*注：具体操作法（手法+穴位）仅列举各种类型便秘频次使用的前 10 项。

4. 中心度分析

如图 3 所示，在不分型便秘的操作法（手法+穴位）网络中，摩腹、推下七节骨、清大肠、揉足三里等

居于操作法网络的中心，中心度较高；推下箕门、揉巨髎等位于操作法网络的外围。

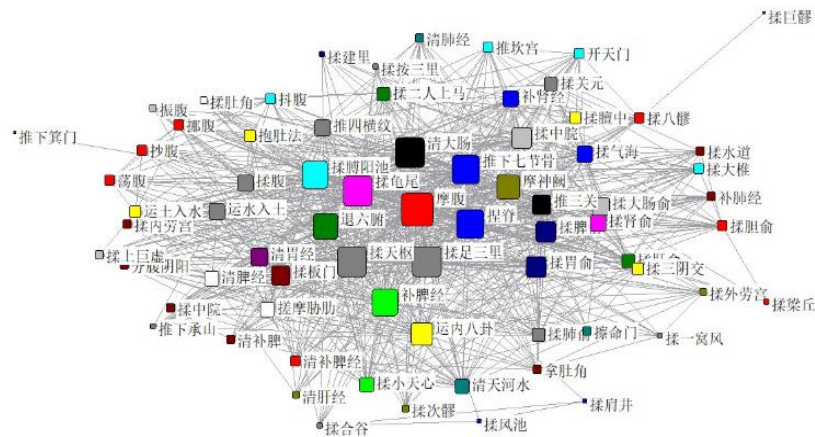


图3 不分型便秘操作法（手法+穴位）网络图

如图4所示，在实秘的操作法（手法+穴位）网络中，清大肠、推下七节骨、摩腹、退六腑等居

于操作法网络的中心，中心度较高；揉小天心、推箕门等位于操作法网络的外围。

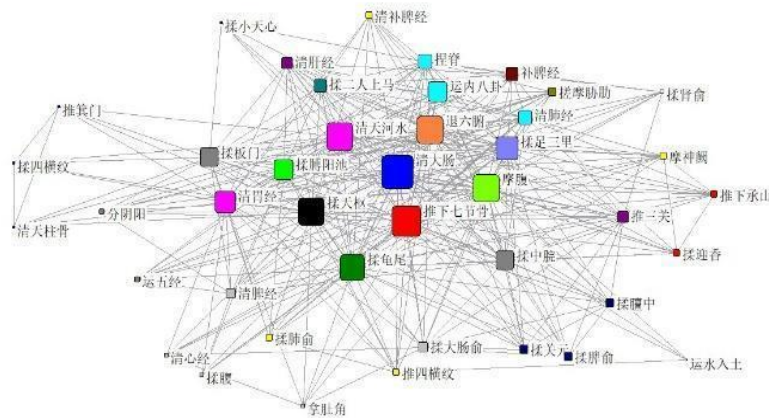


图4 实秘操作法（手法+穴位）网络图

如图5所示，在虚秘的操作法（手法+穴位）网络中，补脾经、捏脊、清大肠、推三关等居操作

法网络图的中心，中心度较高；清肺经、清肝经等位于网络图的外围。

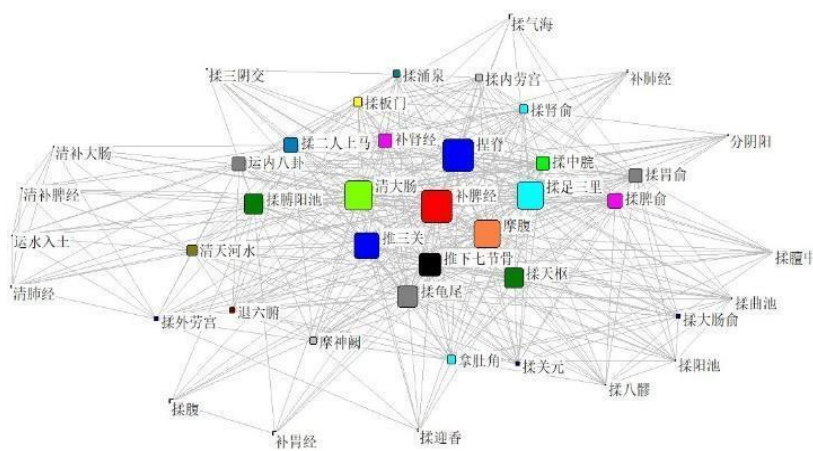


图5 虚秘操作法（手法+穴位）网络图

5. 聚类分析

聚类如图 6、图 7、图 8 和表 2。不分型便秘如图 6 树状图所示，以分组距离 15 为界，可将治疗不分型便秘的高频操作手法分为 3 类，见表 2；实秘如图 7 树状图所示，以分组距离 15 为界，可将治疗实秘的高频操作手法分为 3 类，见表 2；虚秘图 7 树状图所示，以分组距离 15 为界，可将治疗虚秘的高频操作手法分为 3 类，见表 2。

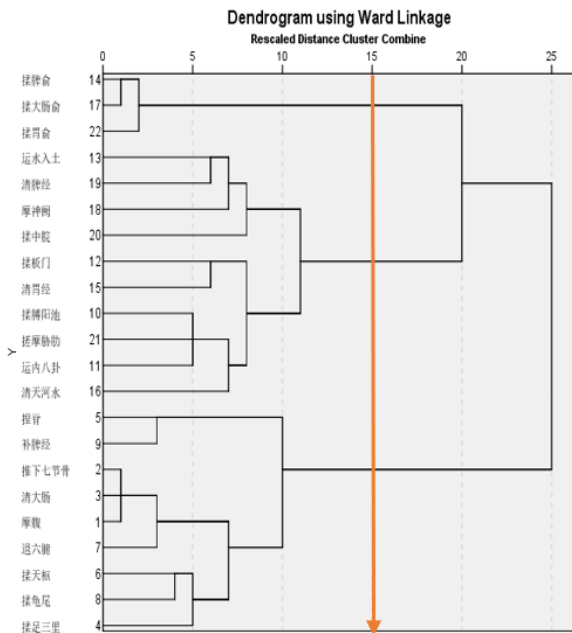


图 6 不分型便秘高频操作法（手法+穴位）聚类树状图

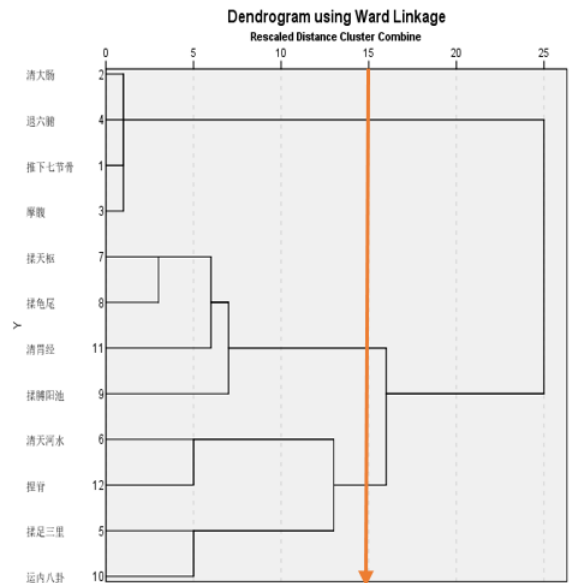


图 7 实秘高频操作法（手法+穴位）聚类树状图

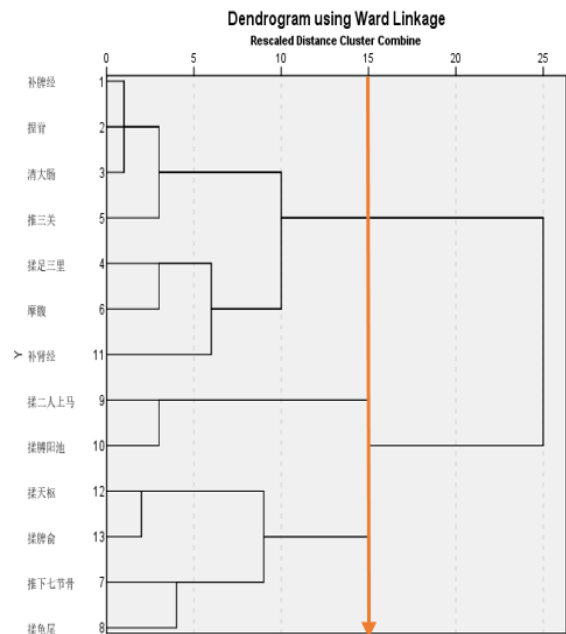


图 8 虚秘高频操作法（手法+穴位）聚类树状图

表 2 聚类分析树状图高频操作法 (手法+穴位)

类型	类别	操作法 (手法+穴位)
不分型便秘	I	摩腹、推下七节骨、清大肠、退六腑、揉天枢、揉龟尾、揉足三里、捏脊、补脾经
	II	运水入土、清脾经、摩神阙、揉中脘、揉板门、清胃经、揉膊阳池、搓摩肋肋、运内八卦、清天河
	III	揉脾俞、揉大肠俞、揉胃俞
实秘	I	清大肠、退六腑、推下七节骨、摩腹
	II	揉天枢、揉龟尾、清胃经、揉膊阳池
	III	清天河水、捏脊、揉足三里、运内八卦
虚秘	I	补脾经、捏脊、清大肠、推三关、揉足三里、摩腹、补肾经
	II	揉二人上马、揉膊阳池
	III	揉天枢、揉脾俞、推下七节骨、揉龟尾

6.推拿操作法 (手法+穴位) 组方

综合小儿推拿治疗儿童便秘操作法 (手法+穴位) 的频次、支持度、中心度, 以及聚类分析, 再结合中医君臣佐使的配伍理论, 可进一步得出小儿推拿治疗儿童便秘操作手法的组方配伍规律 (见表 3), 不分型便秘以摩腹、推下七节骨和清大肠作为君, 退六腑、揉天枢、揉龟尾、捏脊为臣, 补脾经、揉足三里、运水入土、揉板门、摩神阙等操作

法为佐使。实秘以清大肠、推下七节骨、摩腹、为君, 退六腑、揉天枢、揉龟尾、清胃经为臣, 揉膊阳池、捏脊、揉足三里、运内八卦等其他操作法为佐使。虚秘以补脾经、捏脊、推三关为君, 清大肠、揉足三里、摩腹、补肾经为臣, 揉天枢、揉脾俞、揉二人上马、推下七节骨、揉天枢等操作法为佐使。

表 3 小儿推拿治疗儿童便秘的操作法组方, 君臣佐使配伍

类型	君	臣	佐、使
不分型便秘	摩腹、推下七节骨、清大肠	退六腑、揉天枢、揉龟尾、捏脊	补脾经、揉足三里、运水入土、揉板门、摩神阙
实秘	清大肠、推下七节骨、摩腹、退六腑	揉天枢、揉龟尾、清胃经	揉膊阳池、捏脊、揉足三里、运内八卦
虚秘	补脾经、捏脊、推三关	清大肠、揉足三里、摩腹、补肾经	揉天枢、揉脾俞、揉二人上马、推下七节骨、揉天枢

小儿便秘多为糟粕积滞肠腑, 治疗总以“通下”为原则, 故在未予分型的便秘中应以摩腹、推下七节骨、清大肠为君。摩腹, 《理渝骈文》曰: “后天之本在脾胃, 调中者摩腹”, 《厘正按摩要术》[10] 曰: “摩腹用掌心, 团摩满腹上, 治上乳食”, 凡胃肠功能失常引起的各种病症, 均可用摩腹治疗[11]。现代医学也表明, 摩腹可以增加胃肠蠕动, 促进胃肠血液和淋巴循环, 刺激消化液的分泌, 促进食物的消化吸收[12]。推下七节骨, 七节骨位于腰骶部, 名门至尾骨端的连线, 推下七节骨为清、为泄、为降, 可泻热通便[13]。清大肠, 大肠位于食指二节侧, 可清可补, 清大肠可调理大肠, 行气通便, 常用于湿热、食积滞留肠道, 大便秘结等症, 三者皆为清泄导滞之手法。再以退六腑清热解毒、揉天枢

涤肠消滞, 揉龟尾、捏脊通调督脉经气, 温阳健脾为臣; 以补脾经、揉足三里、运水入

土健脾胃, 补气血, 揉板门健脾和胃、消食化滞, 摩神阙温阳散寒, 补益气血为佐使, 此君臣佐使搭配, 符合以通为顺的治疗原则, 同时兼顾患儿气血阴阳的调理。

小儿实秘多由乳食积滞、胃肠燥热、气机郁滞等原因导致, 治疗当以消积导滞、清热通便、疏通气机为主[14]。以清大肠、推下七节骨、摩腹、退六腑为君。再以揉天枢健脾和胃, 理气化滞, 揉龟尾调督温脾、清胃经清泻胃热为臣。以揉膊阳池清泻导滞, 捏脊、揉足三里温阳健脾、补益气血, 运

内八卦行气消滞为佐使，此君臣佐使搭配，符合小儿便秘以清泻通便的治疗原则。

小儿便秘常多因气血不足，肠失濡养，或因阳虚推动无力导致[14]。因小儿脾常不足，而脾胃为气血生化之源，若脾气亏虚，则脾不升清、肠道失润且胃气不降、水谷糟粕传化失司而最终导致便秘的产生[15]，故治疗当以温阳健脾、益气通便。以补脾经、捏脊、推三关为君，补脾经、捏脊、推三关可温阳健脾，促气血化生，清大肠可化积导滞。脾经位于大指端螺纹面，为临床小儿治疗便秘常用穴，脾经的手法主要以补为主，《厘正按摩要术》言：“大指脾胃，宜多补，如热甚，可略泻”。补脾经可促使脾气健行，推动有力，增强肠胃蠕动功能[16]；捏脊疗法最早记述于东晋医家葛洪所著的《肘后备急方》，主要被用来治疗消化系统的疾病，为目前小儿推拿的常用手法。捏脊疗法作用的部位在脊背，起点为长强穴，终点为大椎穴，操作时自上而下刺激督脉、夹脊穴和膀胱经，属温热类手法，能振奋阳气，促进气血的运行，对于小儿虚、寒类的病症有较好的疗效[17]；也有现代研究认为，捏脊疗法可调节血浆内的 Ghrenlin、VIP 水平，改变神经肽物质的浓度，调节胃肠的功能活动[18]，还可调节肠道菌群，增加有益菌群数量，抑制潜在致病菌群的增长，使肠道抵抗力增强，提高机体的免疫力[19]。再以揉足三里健脾益气补血，补肾经补益肾气，清大肠、摩腹理气化滞为臣。以揉脾俞健脾益气，揉二人上马补益肾气，揉天枢、推下七节骨涤肠消滞、泻热通便，为佐使。此君臣佐使搭配，符合小儿便秘以补为主，补中有消，兼调脾肾的治疗原则。

Discussion

便秘首见于《黄帝内经》，又称“大便难”、“阴结”、“阳结”等。《诸病源候论·小儿杂病诸候论》云：“小儿便不通者，脏腑有热，乘于大肠故也”。《外台秘要》又云：“病源大便难者，由五脏六腑不调，阴阳偏有，冷热虚实，三焦不和，则冷热并故也”。便秘的病位在大肠，与其他脏腑，尤其是脾胃的功能失常关系密切[15,20]。根据其临床表现，可分为实秘和虚秘两类[21]。小儿为“稚阴稚阳”之

体，易化寒化热，阻滞气机，津液输布代谢障碍，肠道失去气的推动和津液的濡润而致大便秘结难解，而成实秘[22]。因其“脏腑娇嫩，形气未充”的生理特点，脾胃运化功能尚不成熟，饮食不节则更易损伤脾胃，脾胃损伤则水谷精微腐熟无力，积聚于肠道，气虚大肠传导失司，糟粕蓄积无以排出，形而成虚秘。中医小儿推拿是通过一些特定的手法作用于患儿体表的穴位，以疏通经络，调节机体气血阴阳和脏腑功能，达到预防和治疗疾病的目的[11]。

Conclusion

辨证治疗作为中医的核心特色之一，是保证疗效的一个关键。本文从多角度研究发现，实秘、虚秘的推拿操作具有明显的辨证论治特点，且依据中医君臣佐使的配伍原则，实秘以清泻导滞类为君，虚秘以补益类为君、补泻搭配，体现了中医理论在指导临床实践的相通性和合理性。而未进行辨证论治者以通下为原则，根据各自的兼症，辅以调理阴阳气血的臣和佐使类操作法（手法+穴位），同样起到了较好的疗效。推拿治疗小儿便秘中，是否通过辨证分型的治疗会取得更好的疗效，尚未见报道，有待更多大样本、多中心的 RCTs 进行验证。

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Effects of circRNA in Notch Pathway on Differentiation and Maturation of Oligodendrocyte Progenitor Cells in Cerebral Palsy

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ABSTRACT

CircRNAs are ubiquitous non-coding RNAs, which are richly expressed in brain tissues and participate in the development and signaling of the nervous system and regulate complex functions and various neural activities of the brain. The Notch pathways can regulate multiple processes of cell morphology by regulating cell damage and apoptosis, inflammation, nerve repair and angiogenesis, and other processes involved in the occurrence and development of diseases. The Notch pathway critically regulates the differentiation and maturation of oligodendrocyte progenitor cells (OPCs), and the abnormal expression of circRNA can regulate the differentiation and maturation of OPCs through many pathways, including the Notch pathway, thus participating in the occurrence and development of cerebral palsy. This paper systematically reviewed the effects of circRNA on the differentiation and maturation of OPCs in cerebral palsy by regulating the Notch pathway to provide new ideas for the diagnosis, treatment, and research of cerebral palsy.

Keywords: *CircRNA; Notch; Cerebral palsy; OPCs differentiate and mature*

Introduction

脑性瘫痪 (Cerebral Palsy, CP), 简称脑瘫, 是一组持续存在的中枢性运动和姿势发育障碍、活动受限症候群。其运动障碍常伴有感觉、知觉、认知、交流和行为障碍, 以及癫痫和继发性肌肉、骨骼问题 [1]。据文献报道, 中国0~6岁儿童脑瘫患病率约为0.248%, 其中男童脑瘫患病率为0.22%, 女童脑瘫患病率为0.12% [2,3], 发达国家脑瘫的发病率在2.11‰ [4]。CircRNA分子呈封闭环状结构, 不受RNA外切酶影响, 表达更稳定, 不易降解 [5]。CircRNA在脑组织中表达丰富, 参与神经系统发育和信号传递, 调控大脑复杂的功能和多种神经活 [6]。而Notch通路是决定细胞命运的关键通路, CircRNA与Notch通路可能在CP的发生与发展中起到重要的调控作用。

CircRNA的作用及在神经系统中的应用

1. CircRNA简介及作用

人类基因组在转录水平上极为活跃, 约1.9%的基因序列被转录为蛋白质, 其他则被转录为非编码RNA [7]。CircRNA是一类参与多种生物学过程的非编码RNA, 没有5'帽和3'尾, 是mRNA反向剪接产生的共价闭合RNA分子, 根据基因组的起源和产生方式, CircRNA大致可以分为三种类型: 内含子环状CircRNA、外显子环状CircRNA和外显子内含子环状CircRNA [8]。CircRNA是真核生物中共价封闭的内源性生物分子, 且具有丰富的miRNA结合位点, 可充当miRNA或蛋白质“海绵”; 与蛋白结合, 调控蛋白活性; 参与蛋白翻译 [9-11], 以及miRNA对其靶基因的调控作用 [12]。

2. CircRNA在神经系统中的应用

2.1 CircRNA参与神经系统的发育。

在大鼠发育时间点包括幼年(2周)、青春期(6周)、成年(21周)和老年期(104周)对大脑、心脏、肺、肝脏、肾脏、肌肉、睾丸和胸腺的circRNA进行检测,发现在大脑中有更高的丰度和多样性,其次是肺和胸腺。幼年大鼠产生的CircRNA水平最低,随后在发育过程中呈上升趋势,脑CircRNA宿主基因在神经递质分泌、突触活动和神经元成熟中富集[13]。且在突触、突触前活性区、突触前膜等与突触功能相关的功能群明显富集,通过定量PCR技术明确了CircRNA的表达水平受神经可塑性的调节[14]。

2.2 CircRNA参与调控神经系统疾病

circRNA在人类大脑和视网膜中高度代表的一种是ciRS-7 (CDR1as),低CDR1as水平可导致miR-7表达增加,从而下调阿尔茨海默病 (Alzheimer's disease, AD)的重要靶点UBE2A的活性,其功能参与清除AD脑中的毒性淀粉样肽,进而影响疾病的进程[15]。Ma N [16]发现AD小鼠模型中CircRNA相关的ceRNA网络主要参与树突发育与记忆 (Sorbs2)和小鼠神经发育 (ALS2)。急性缺血性脑卒中患者血浆中CircSCMH1水平明显降低, CircSCMH1增强了神经元可塑性,抑制了神经胶质激活和外周免疫细胞浸润,可促进小鼠和猴子中风后的功能恢复[17]。Chen W [18]通过上调CircUCK2水平可显著减少缺血性脑中风的梗死体积,减轻神经元损伤,改善神经功能缺损,且发现CircUCK2作为内源性miR-125b-5p海绵抑制miR-125b-5p活性,导致生长分化因子11 (GDF11)表达增加,进而改善神经元损伤。mmu-CircRNA-015947参与脑缺血再灌注损伤也说明Circ RNA参与神经系统发育和信号传导[19]。

CP作为儿童神经系统中常见的病症之一,也是儿童严重神经残疾最常见的原因,CP的高致残性和防治困难的特点,取决于其病因及病理生理机制的多样性和复杂性。CP的主要生理病理病机是少突胶质细胞 (oligodendrocytes,OLs)发育不良、脱髓鞘、损伤和变性引起的脑室周围白质损伤。CircRNA在CP中的异常表达为阐明CP发生发展机制和探明早期干预靶点提供了条件。本文重点探讨CircRNA通过CircRNA-miRNA-mRNA轴在Notch通路中对CP的少突胶质前体细胞 (oligodendrocyte precursor cells, OPCs)分化和成熟的影响。

Notch通路及其与CP的联系

1. Notch通路及其信号因子的作用机制

Notch通路是通过细胞与细胞之间的通信实现的,即一个细胞上的跨膜配体激活相邻细胞上的跨膜受体。首次研究来源于约翰·德克斯特和托马斯·亨特·摩根观察到翅膀有凹槽的突变果[20]。迄

今为止,已经确定了信号转导级联的主要组成部分和步骤,明确了Notch通路能够在大量的细胞类型中决定细胞命运。哺乳动物表达四种高度同源受体 (Notch 1、2、3和4)和五种典型配体 (Delta-like 1、3、4和Jagged 1、2),受体和配体都是跨膜蛋白。经典的Notch通路,即信号从Jagged/Delta like配体与Notch受体相互作用到受体切割[21],3次蛋白水解步骤产生一个活化的Notch片段[22]。第1次蛋白裂解步骤(S1裂解)由Furin介导,第2次裂解(S2裂解)由 γ -分泌酶介导的, γ -分泌酶在表达Notch的细胞中第3次裂解靠近膜内小叶(S3裂解)的栓系受体,产生具有转录活性的NICD, NICD进入细胞核,与转录因子相互作用形成转录激活复合物,激活下游靶基因转录[23,24]。

Hes1、Hes5、Hey1、Hey2和HeyL是Notch信号通路的靶基因[25]。Hes基因属于抑制型碱性螺旋-环-螺旋(basic helix-loop-helix,bHLH)家族,不仅能够调节中枢神经系统的功能,而且对胶质细胞的形成至关重要[26]。Ohtsuka等[27]通过逆转录病毒错误表达Notch (caNotch)在野生型、Hes1缺失、Hes5缺失和Hes1-Hes5双缺失的小鼠胚胎,发现诱导内源性Hes1和Hes5表达的caNotch在野生型、Hes1缺失和Hes5缺失背景下抑制了神经元分化,而在Hes1-Hes5双缺失背景下则没有,说明Hes1和Hes5是调控哺乳动物神经分化的重要Notch效应因子。

1.1 Notch通路在神经系统疾病中的作用

在斑马鱼中发现Notch通路与其中枢神经胚胎髓鞘缺陷的相关性[28]。多发性硬化症中以配体依赖性Notch信号通路促进再髓鞘化[29]。蛋白激酶R活化因子与C启动子结合因子1 (CBF1)相互作用,并显著增强CBF1与Notch胞内区域(NICD)之间的关联,证明Notch通路的相关转录因子,在哺乳动物大脑发育过程中发挥着关键作用[30]。中枢神经系统感染小鼠中,下调miR-25可能通过激活Notch通路提高中枢神经系统感染小鼠的记忆能力[31]。人类特异性Notch2NL的三个平行序列在神经胶质细胞中高度表达,功能分析表明Notch2N异位表达推迟了神经元祖细胞的分化,而其缺失则加速了皮层神经元的分化[32]。Notch通路广泛参与神经系统疾病,低氧/缺血状态激活Notch通路,可通过低氧/缺血预处理的方式调控Notch通路从而起到神经保护的作用。

2. Notch通路与CP的联系

2.1 CP的病因及发病机制

CP的病因和发生机制复杂,可概括为宫内、围产期因素及新生儿脑室内出血、脑室周围白质软化、脓毒症和新生儿中风等[33]。CP的脑部病理改变主要是脑白质损伤、脑部发育异常、颅内出血、脑部缺氧引起的脑损伤。根据临床表现,CP又可分为痉挛型、不随意运动型、强直型、共济失调型

、肌张力低下型、混合型共6种分型[1]，其中以痉挛型多见，占85%-91% [34]。脑室周围白质损伤是痉挛型CP的常见原因，磁共振成像可显示脑室周围白质区异常信号和/或体积损失、侧脑室增大、胼胝体变薄 [35]，而CP患者常伴有白质结构连接异常 [36]。白质是皮层下的重要组成部分，是分布式神经网络中信息传递的提供者。白质束约占整个脑容量的一半，贯穿整个大脑，其补充了大脑皮层的信息处理，提供了大脑内部的信息传递，使神经系统具有快速、有效的整合能力 [37]。

正常的脑白质主要由大量的神经元轴突和被少突胶质细胞OLs包绕形成的髓鞘组成 [38]。OLs在中枢神经系统轴突周围生成多层髓鞘膜，以实现快速有效的神经传导，OLs既可形成髓鞘，又可为轴突提供代谢支持 [39,40]，但极易受到氧化损伤 [41]。缺血缺氧等因素会阻止OLs及髓鞘发育 [42]。脑室周围白质软化 (periventricular leukomalacia, PVL) 被定义为脑深部白质损伤，是脑室周围白质损伤的经典病理表现，以侧脑室旁白质深部坏死灶为特征，严重的情况下，可延伸至半卵圆中心，甚至皮质下白质。PVL的主要特征是一种慢性髓鞘形成障碍，提示OLs谱系进展被缺血损伤所破坏 [43]。OLs谱系是从OPCs分化到Pre-OLs，再到成熟OLs，最后到完全成熟OLs的过程，在小鼠中大多数OLs产生于出生后的前4周，新的OLs的产生持续到整个成年期，尽管这一过程随着年龄的增长而下降，但还受到诸如日光或体育锻炼等环境因素的影响 [44]。研究发现PVL与OLs谱系密切相关，脑室周围顶叶白质是脑室周围白质软化的高易感区域，主要在妊娠24-32周，此阶段是PVL的高发时期 [42]。可见在发育过程中，OPCs最终分化和成熟为构成髓鞘的OLs，在促进神经元信号传导中发挥重要作用 [44,45]。

2.2 OPCs的分化和成熟是CP脑白质修复的关键

OPCs起源于胚胎脑和脊髓的脑室区，从这些区域OPCs通过中枢神经系统广泛迁移，实现均匀分布 [46]。成人中OPCs约占中枢神经系统胶质细胞总数的5-8%，在脱髓鞘反应中，OPCs分裂和分化成新生OLs取代已丢失的OLs。OPCs在中枢神经系统中含量丰富，可分化为OLs直至其成熟，以适应髓鞘形成和损伤或疾病后髓鞘再生的能力[47]。Kirby [48]发现 OPCs可在多发性硬化症中经免疫系统增持以维持自身的免疫反应，这提示可通过抑制OPCs的免疫激活从而促进髓鞘再生。Huang W [49]研究发现OPCs可在相同时间内对称分裂，数量上以指数方式产生子代细胞以扩增祖细胞池，其子代细胞互相远离，呈放射状向相反方向迁移以确保可广泛分布于脑皮层，这表明OPCs的增殖和分散特性对白质扩张和髓鞘形成非常重要。

脑室周围白质损伤扰乱了正常的OPCs分化进程和白质发展中的髓鞘化 [43]。许多创伤、损伤和感染

都可触发OLs死亡，造成脱髓鞘从而导致功能紊乱。脱髓鞘诱导轴突缺陷，如节点复杂改变、传导阻滞和/或不可逆的轴突丢失，明显破坏远程连通性，这些障碍共同导致运动、感觉和认知功能障碍 [50]。Hesp等 [51]发现在脊髓损伤后1-2天分裂的OPCs产生的OLs主要分布在胶质瘢痕或病变边缘和病变周围的剩余组织中，相比之下，4周分裂的OPCs产生的新OLs分布更为广泛，主要位于靠近脑顶边缘的远端备用白质中，以重新形成未受损的轴突。斑马鱼身上使用活体成像显示OLs在体内非常短的时间窗 (约5小时) 内可生成新的髓鞘 [52]。Keirstead研究 [53]提示髓鞘再形成的失败可能是由于OPCs的减少和耗尽。OPCs向OLs的分化和髓鞘形成不仅受时间和空间的调控 [41]细胞内的转录因子、表观遗传调节因子、DNA甲基化、非编码RNA和信号通路都参与调节其分化和成熟 [45]。

2.3 Notch通路可调控OPCs的分化和成熟

Zhou H [54]通过脑瘫幼鼠测序发现Notch通路与脑瘫密切相关。而脑白质变性的患者，磁共振成像在T2加权图像中显示多个高强度的白质病变，甚至在临床症状出现前10-15年，基因检测发现其Notch3基因发生突变。多年后其女儿基因检测也发现Notch3基因出现异常 [55]。Gao L [56]发现在新生儿缺血缺氧性脑损伤模型中，与假手术组相比，实验组中Hes1蛋白和mRNA的表达增加，且通过TWS119调控Notch通路在缺血缺血性脑损伤后7天实现神经保护作用。体外试验 [57]表明细胞缺血缺氧可上调Notch信号通路中Notch1、Hes1和Hes5的表达。此外，有研究发现使用Notch抑制剂DAPT后抑制了OPCs的增殖和分化 [58]。Fbxw7通过调控Notch通路限制斑马鱼OPCs的数量 [59]。内皮素-1以自分泌的方式通过调控Notch通路促进星形胶质细胞的维持和增殖 [60]。

CircRNA 通过CircRNA-miRNA-mRNA轴在Notch通路中对CP的作用

1. CircRNA/miRNA对CP的影响

CP中异常的CircRNA可能参与了其发病机制。Liu CY [61]对60例新生儿缺氧缺血性脑病患者检测血清中miRNA-373、缺氧诱导因子 (HIF-1 α)、基质金属蛋白酶9 (MMP-9) 和血管内皮生长因子 (VEGF) 的表达，结果显示miRNA-373与三者都存在很强的关系，且实验组中miRNA-373、HIF-1 α 、MMP-9、VEGF表达水平明显高于对照组。在CP模型中，过表达miR-146b-5p可显著抑制缺血缺氧诱导的PC12细胞损伤、炎症反应和氧化应激从而减轻缺血缺氧诱导的神经元损伤 [62]。细胞在髓鞘培养基培养14天发现，miR-219/miR-338处理后的培养基中成熟OLs的数量变多、髓鞘长度增加和髓鞘结构更完整，表明miR-219/miR-338增加了OLs的分化率和分化程度，参与髓鞘化过程 [63]。

2. CircRNA/miRNA与Notch通路的联系

Zhao X等[31]通过转染具有miR-219和/miR-338模拟物的原代少突胶质细胞前体细胞培养物4天,发现Hes5的mRNA表达显著降低,同时在成年海马源性神经祖细胞中Hes5表达同样大幅降低;相反,通过miRNA抑制剂敲低miR-219和/miR-338,导致原代少突胶质细胞前体培养中Hes5表达上调,这些观察结果表明,Hes5的表达受到miR-219和miR-338的负调控。Circ_0111277在滋养层细胞中吸附hsa-miR-494-3p,通过调控HTRA1/Notch-1上调hsa-miR-494-3p的表达水平[64]。与正常脑组织相比,Notch信号在肿瘤组织中显著上调,CircNFIX充当了miR34a-5p的海绵,这是一种靶向Notch1的miRNA。下调CircNFIX和上调miR34a-5p均可抑制细胞增殖和迁移。此外,miR34a-5p抑制剂可中和CircNFIX对胶质瘤细胞的抑制作用。在体内实验中,CircNFIX通过调控miR34a-5p和Notch1抑制胶质瘤的生长[65]。精神活性大麻素和非精神活性大麻二酚可以通过降低脂多糖上调的Notch配体Dll1的表达,被调控的miRNA及其靶基因受到Notch等交叉信号控制,参与免疫应答、细胞周期调控以及细胞应激和氧化还原稳态[66]。

CircRNA在神经系统中作用机制的研究发展迅速,随着测序技术和生物学技术迅速发展,CircRNA的功能将越来越多的被揭示出来。大鼠胚胎中分离出的皮质神经干细胞经培养后发现,CircRNA 9-cis-RA可显著降低Notch通路转录因子Hes5的表达从而促进OPCs的分化[67]等研究,可证明Notch通路在CP的发生发展过程中确实发挥着不可或缺的作用,通过CircRNA-miRNA-mRNA轴探索CircRNA与Notch通路参与调控CP的作用机制值得深入。

Conclusion

脑瘫的病因病机复杂,是临床及基础研究的重点及难点。近年来对Notch信号通路的研究中已经发现了其对中枢神经系统中的重要作用,主要是通过调控细胞形态的多个发生过程所造成神经系统的损伤。因此通过调控Notch通路抑制细胞损伤及凋亡、炎症、神经修复和血管再生等过程,成为临床防治脑瘫疾病的重要途径。CircRNA通过CircRNA-miRNA-mRNA轴影响脑瘫的发生发展过程的报道也备受关注,尽管愈来愈多的证据表明CircRNA在神经系统中重要作用,但CircRNA相互之间复杂的分子机制及相关通路尚未完全了解。将CircRNA作为靶点进行深入的实验研究,分析其上、下游的分子调节机制,可为阐明脑瘫的发生机制提供理论依据,并且为脑瘫的诊断和治疗方法提供新思路。

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Clinical Observation of Magui Wenbi Granules in the Treatment of Rheumatoid Arthritis and Its Effect on IL-23 / IL-17 Axis

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ABSTRACT

Introduction: Maguiwenbi granules are modified from Professor Wang Lvqiu's Xinbitongling granules used to treat cold-damp arthralgia syndrome in active RA and achieved an excellent curative effect. Previous studies have found that Xinbitongling can inhibit the expression of RANKL and IL-17 in the serum of CIA rats and alleviate the bone destruction of RA.

Objective: To observe the clinical efficacy of Magui Wenbi Granules in treating active rheumatoid arthritis (cold-dampness syndrome).

Methods: 60 patients were collected according to the NAPA standard and randomly divided into a control group and a treatment group with 30 cases each. The control group received conventional treatment, and the treatment group was added with Maguiwenbi granules. The joint treatment period was 12 weeks. Record the number of joint tenderness, joint swelling, DAS28, ESR, CRP, RF, TCM syndrome score before and after treatment, serum IL-23 and IL-17A levels, and use TCM syndrome efficacy evaluation standard and ACR20/50/70 standard to evaluate Perform overall clinical efficacy.

Results: 59 patients, 1 excluded, 29 in the treatment group, and 30 in the control group. (1) The treatment group was significantly better than the control group in reducing the number of joint tenderness, swollen joints, DAS28, ESR, and TCM syndrome scores ($P < 0.01$), and better than the control group in reducing IL-17A ($P < 0.05$). However, there was no statistical difference between the two groups after treatment; (2) The total effective rate of TCM syndromes in the treatment group was 96.6%, and in the control group was 70.0%; the ACR20 and ACR50 of the treatment group were 96.6% and 24.1%, respectively, the ACR20 and ACR50 of the control group were 73.3% and 20.0%.

Conclusion: Maguiwenbi Granules combined with conventional regimens have a definite clinical effect in the treatment of RA. It can significantly relieve the patient's clinical symptoms, reduce disease activity, increase clinical remission rate, and reduce IL-23 and IL-17A levels. It may inhibit IL-23/IL-17 inflammation axis.

Keywords: rheumatoid arthritis; cold dampness and stasis syndrome; Magui Wenbi granule; clinical observation; IL-23/IL-17 axis

Introduction

类风湿关节炎 (rheumatoid arthritis, RA) 是一种慢性、进行性、系统性的自身免疫性疾病 [1]。近年来类风湿关节炎发病机制的热点集中在调节性T细胞 (Treg) 和辅助性T细胞 (Th, 主要是Th17细胞) 之间免疫平衡打破后的免疫调节异常。Treg 细胞与 Th17 细胞在分化与发育中, 存在着一种相互约束的作用。当疾病发生时, 细胞分化与增殖倾向 Th17 细胞, 其数

量增多, 反之 Treg 细胞的数目减少, 最终引发 Th17 与 Treg 之间的平衡失调。IL-23 在 Th17 细胞的分化和发育中起了重要作用。Th17 需在 IL-23 诱导下游靶基因后方能增殖并激活其对 IL-17 的分泌, 维持和放大 IL-17 的功能, 进一步激活通路, 诱导产生下游炎症细胞因子, 以上形成 IL-23/IL-17 炎症轴。当免疫平衡的天平倾向 Th17 后, 会诱导大量炎症细胞因子分泌, 相关因子介

导的多种细胞及酶类的释放，造成滑膜炎、关节破坏、骨侵蚀等，加速RA的进展 [2-4]。

麻桂温痹颗粒一脉相承汪履秋教授的新痹痛灵颗粒，汪悦教授将其长期运用于RA活动期寒湿痹阻证的治疗，并取得较好的疗效。前期研究发现本方能够抑制CIA大鼠血清中RANKL和IL-17表达，缓解RA骨破坏 [5]。本文通过观察麻桂温痹颗粒治疗 RA 活动期寒湿痹阻证的疗效，及本方对 IL-23/IL-17 炎症轴的调节作用，为后期进一步研究其作用机制提供基础。

Methodology

1. 资料

1.1 病例来源

2018.12-2020.03 南京中医药大学附属医院风湿免疫科门诊及住院患者 60 例，采用随机分组原则，经过样本量估算及临床收集病例数实际情况，将纳入患者分为治疗组 30 例和对照组 30 例，最终完成治疗组 29 例，男 5 例，女 24 例，平均年龄 51.41 ± 12.53 岁，病程 $59.0 (27.5, 101.0)$ 月，对照组 30 例，男 5 例，女 25 例，平均年龄 56.67 ± 11.70 岁，病程 $74.5 (30.5, 135.0)$ 月。两组资料比较差异无统计学意义，具有可比性。

1.2 纳入与诊断标准

(1) 符合 2010 年 ACR/EULAR 类风湿关节炎诊断标准 [6] (2) 中医寒湿痹阻证辨证标准 [7]。符合活动期 RA 标准。(3) 年龄在 18~80 岁之间，性别不限。

1.3 排除标准

(1) 不符合纳入标准者。(2) 重叠其他风湿病。(3) 长期服用其他治疗类风湿关节炎的中西药物，且难以减停者。(4) 妊娠期或哺乳期妇女。

(5) 过敏体质，如对中药过敏者。(6) 合并有严重的心脑血管病变、内分泌系统病变、消化系统病变、血液系统病变、神经系统病变等。(7) 合并严重畸形疾病或者丧失劳动力者。(8) 可能存在或确实有酒精、药物滥用史。(9) 根据研究者判断，易造成失访者。

1.4 病例剔除及脱落标准

(1) 受试者依从性差，或中途自行换药或加用其他药物。(2) 随访期间不能完成观察指标检测或无检测记录可评价者。(3) 因药物副反应不能继续原治疗方案者。(4) 观察中自然脱落、失访者。

2. 方法

2.1 治疗组

麻桂温痹颗粒（每日一剂，早晚两次，颗粒冲服 300mL）+甲氨蝶呤片 10mg 口服 qw+塞来昔布 200mg 口服 qd 治疗。中药药物组成：麻黄，桂枝，防风，防风等 6 味中药颗粒（江阴天江药业有限公司生产）对照组：甲氨蝶呤片 10mg 口服 qw + 塞来昔布 200mg 口服 qd 治疗。治疗疗程：二组均连续服药 12 周。临时给药：6 周后 VAS 评分 > 8 分，酌情临时加量塞来昔布至 200mg 口服 bid。不使用与本病治疗相关的其他药物。

2.2 观察指标

采集的血样先在 4℃ 静置 2h，2500r/min 离心 10min，取上清液 -80℃ 保存，ELISA 法测定血清 IL-23、IL-17A 的水平；压痛关节数、肿胀关节数、DAS28、血沉 (ESR)、类风湿因子 (RF)、C-反应蛋白 (CRP)。中医证候积分、中医疗效评定标准、ACR20/50/70 标准，记录一般生命体征、血、尿常规、肝肾功能、心电图等。记录不良反应的症状、发生时间、发生频次等，首先采取对症治疗，如症状严重不得缓解是应当立即停止本次试验。

3. 观察指标与方法

3.1 临床观测指标

(1) 相关症状体征：压痛关节数、肿胀关节数、VAS 评分、DAS28、患者生活能力自我评价量表 (HAQ) (2) 临床指标：血沉 (ESR)、类风湿因子 (RF)、C-反应蛋白 (CRP)。

3.2 中医证候积分

参照《中药新药临床研究指导原则》[7]2002 年“中药新药治疗类风湿关节炎的临床研究指导原则”的症状分级量化拟定

3.3 总体疗效评定

3.3.1 中医疗效评定标准

参照《中药新药临床研究指导原则》[7]“中药新药治疗类风湿关节炎的临床研究指导原则”。综合疗效指数 = (治疗前积分 - 治疗后积分) ÷ 治疗前积分 × 100%。A 临床痊愈：临床症状、体征消失或基本消失，证候积分减少大于或等于 95%；B 显效：中医临床症状、体征明显改善，证候积分减少大于或等于 70%；C 有效：中医临床症状、体征均有好转，证候积分减少大于或等于 30%；D 无效：中医临床症状、体征均无明显改善，证候积分减少小于 30%。

3.3.2 ACR20/50/70 标准

参照 ACR 反应标准：1. 关节压痛数；2. 关节肿胀数；3. 患者对疼痛的评价；4. 患者对疾病活动性的综合评价；5. 医生对疾病活动性的综合评价；6. 患者对生活能力的自我评价 (HAQ)；7. 急性炎症反应物 (ESR)。

ACR20 标准：满足 1 和 2 均 ≥ 20%，同时 3~7 中至少满足三项指标改善 ≥ 20%。

ACR50 标准：满足 1 和 2 均 ≥ 50%，同时 3~7 中至少满足三项指标改善 ≥ 50%。

ACR70 标准：满足 1 和 2 均 ≥ 70%，同时 3~7 中至少满足三项指标改善 ≥ 70%。

指标改善百分率 = [(治疗前值 - 治疗后值) ÷ (治疗前值)] × 100%

3.4 IL-23、IL-17A 水平

两组患者治疗前后使用 ELISA 法测定血清 IL-23、IL-17A 的水平，对实验数据进行统计学分析。

3.5 试剂及仪器

3.5.1 ELISA 试剂盒 (MULTI SCIENCES)；Infinite M200 Pro 多功能酶标仪 (CHETECAN)；孵箱 (USA Thermoprecision)；-80℃ 超低温冰箱 (JP SANYO)；Centrifuge 5427R 24 孔台式高速冷冻离心机 (GER Eppendorf)。

3.5.2 标本的采集、保存

采集外周静脉血 3ml，离心后，吸出上层血清，置于-80 °C冰箱保存。

3.5.3操作步骤

(1) 试剂、样品平衡至室温。(2) 准备试剂及标准品。(3) 浸泡酶标板：加入 300μl 洗液静置浸泡半分钟。(4) 加标准品：加入100μl 2倍比稀释的标准品。(5) 加样：加入50μl 样本和50μl 检测缓冲液。(6) 加抗体：每孔加入 50μl 稀释的检测抗体 (1:100稀释)。(7) 孵育：封板膜封板。300转/分钟振荡，室温孵育2小时。(8) 洗涤：每孔加洗涤液洗涤6次，拍干。(9) 加酶：每孔加入 100 μl 稀释的辣根过氧化物酶标记的链霉亲和素 (1:100 稀释)。(10) 孵育：重复步骤 (7)，孵育45分钟。(11) 洗涤：重复步骤 (8)。(12) 显色：每孔加入 100μl 显色底，避光孵育5-30分钟。(13) 终止：每孔加入100μl 终止液。(14) 读数：使用酶标仪进行双波长检测，测定450nm和630nm参考波长下的OD值 (15)：计算标准品和样本的平均 OD 值，然后减去零浓度标准品的 OD

值。通过对浓度值和OD值取对数拟合，可以对标准曲线进行线性化。

4. 统计学方法：

采用 SPSS 25.0 对数据进行统计学分析。计量资料若符合正态分布，采用 t 检验，用均数±标准差 ($\bar{x} \pm s$) 表示；若不符合正态分布，采用非参数检验，用中位数 (四分位数间距) (Md(IQR))表示。P<0.05 为差异有统计学意义，P<0.01 为差异具有显著统计学意义。

Results

1. 压痛及肿胀关节数

治疗后两组压痛关节数较前明显减少，差异具有显著统计学意义 (P<0.01)。治疗后两组肿胀关节数较前明显减少，差异具有显著统计学意义 (P<0.01)。治疗后治疗组压痛、肿胀关节数较对照组减少，差异具有统计学意义 (P<0.05)。表明经两组均能明显降低关节压痛数和关节肿胀数，且治疗组优于对照组。见表1

表1两组治疗前后压痛关节数及肿胀关节数比较(Md(IQR))

组别	压痛关节数		肿胀关节数	
	治疗前	治疗后	治疗前	治疗后
治疗组 (N=29)	8(6,10)	3(3,3.5) ^{△△▲}	6(3.5,7)	1(1,2) ^{△△▲}
对照组 (N=30)	6.5(4,10)	5(2,7.25) ^{△△}	3.5(2,8)	2(1,5.25) ^{△△}
<i>P</i>	0.271	0.045	0.247	0.046

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.001

2. DAS28 评分

两组治疗后 DAS28 评分较前明显降低，差异均具有显著的意义 (P<0.01)。治疗后治疗组较对照组降低，差异具有显著的意义 (P<0.01)。表明两组均能明显降低患者的 DAS28 评分，且治疗组明显优于对照组。见表2

表2 两组治疗前后DAS28比较($\bar{x} \pm s$)

组别	DAS28	
	治疗前	治疗后
治疗组	5.22±0.58	3.18±0.57 ^{△△▲▲}
对照组	4.92±0.95	4.01±0.91 ^{△△}
<i>P</i>	0.15	0

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.01

3. ESR、CRP、RF

两组治疗后 CRP 较前降低，差异具有统计学意义 (P<0.05)。表明两组均能降低患者CRP。见表3

表3两组治疗前后CRP比较(Md(IQR))

组别	CRP(mg/L)	
	治疗前	治疗后
治疗组 (N=29)	10.30(3.69,18.00)	5.60(1.88,7.82) [△]
对照组 (N=30)	10.30(6.89,17.53)	6.30(3.21,10.30) [△]
<i>P</i>	0.564	0.192

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.01

两组治疗后 ESR 较前均明显降低，差异具有显著统计学意义 (P<0.01)。表明两组均能降低患者 ESR，且治疗组明显优于对照组。见表4

表4两组治疗前后ESR比较(Md(IQR))

组别	ESR(mm/h)	
	治疗前	治疗后
治疗组(N=29)	41.41±21.24	14.52±7.38 ^{△△▲▲}
对照组(N=30)	38.77±29.14	28.87±18.11 ^{△△}
P	0.625	0

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.01 治疗后治疗组较前均明显降低，差异具有显著统计学意义 (P<0.01)，对照组较前降低，差异具有统计学意义 (P<0.05)。表明两组均能降低RF，差异不具有统计学意义。见表5

表5两组治疗前后RF比较($\bar{x}\pm s$)

组别	RF(U/L)	
	治疗前	治疗后
治疗组(N=29)	86.59±46.52	51.05±33.73 [△]
对照组(N=30)	84.97±47.83	66.38±60.93 ^{△△}
P	0.895	0.33

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.01

4. IL-23、IL-17A 水平

治疗后治疗组 IL-23 水平较前降低，差异具有统计学意义 (P<0.05)，IL-17A 水平较前明显降低，差异具有显著统计学意义 (P<0.01)；治疗后对照组 IL-23 水平较前差异无统计学意义 (P>0.05)，IL-17A 水平较前明显降低，差异具有统计学意义 (P<0.01)；治疗后治疗组较对照组 IL-17A 水平降低，差异具有统计学意义 (P<0.05)。表明治疗组能降低 IL-23 水平，优于对照组。两组均能明显降低 IL-17A 水平，且治疗组优于对照组。见表6

表6两组治疗前后IL-23、IL-17A比较($\bar{x}\pm s$)

组别	IL-23(pg/ml)		IL-17A(pg/ml)	
	治疗前	治疗后	治疗前	治疗后
治疗组(N=29)	26.62±7.32	22.49±4.72 [△]	14.26±4.42	8.87±4.15 ^{△△▲▲}
对照组(N=30)	25.59±5.00	24.86±5.44	14.85±4.99	11.31±4.84 ^{△△}
P	0.532	0.052	0.634	0.044

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；两组间比较，[▲]P<0.05，^{▲▲}P<0.01

5. 中医证候积分

两组患者治疗前后中医证候积分经正态性检验，不符合正态分布，采用秩和检验。治疗后两组中医证候积分较前均明显减少，差异具有显著统计学意义 (P<0.01)。治疗后治疗组对照组明显降低，差异具有显著统计学意义 (P<0.01)。表明两组均能明显降低中医证候积分，治疗组明显优于对照组。见表7

表7两组治疗前后中医证候积分的比较(Md(IQR))

组别	中医证候积分	
	治疗前	治疗后
治疗组(N=29)	16.0 (13.5,18.0)	6.0 (4.0,10.0) ^{△△▲▲}
对照组(N=30)	17.0 (13.5,18.0)	11.0 (8.0,13.0) ^{△△}
P	0.848	0

*注：与本组内比较，[△]P<0.05，^{△△}P<0.01；与对照组比较，[▲]P<0.05，^{▲▲}P<0.01

6. 中医证候疗效评定标准

治疗后治疗组1例临床缓解，16例显效，11例有效，1例无效，总有效率为93.1%；对照组无临床缓解病例，5例显效，16例有效，9例无效，总有效率为86.7%，总有效率两者差异具有显著统计学意义 (P<0.01)。表明治疗组中医证候临床疗效明显优于对照组。见表8

表8两组患者中医证候疗效比较

组别	临床缓解	显效	有效	无效	总有效率
治疗组 (N=29例)	1(3.4%)	16 (55.2%)	11 (37.9%)	1 (63.4%)	28 (96.6%)
对照组 (N=30例)	0 (0%)	5 (16.7%)	16 (53.3%)	9 (30.0%)	21 (70.0%)
χ^2	7.680				
P	0.006				

7. ACR20/50/70 标准

治疗后治疗组符合 ACR20 标准 28 例，ACR50 标准 7 例，未有符合 ACR70 病例；对照组符合 ACR20 标准 22 例，ACR50 标准 6 例，未有符合 ACR70 标准的患者；治疗组符合 ACR20 标准高于对照组，差别具有统计学差异 (P<0.05)，ACR50、ACR70 两组之间的差异不具有统计学意义 (P>0.05)。表明治疗组在 ACR20 标准上优于对照组，达到 ACR50/70 标准上差异尚不具有统计学意义。见表 9

表9两组患者ACR20/50/70疗效的比较

组别	ACR20	ACR50
治疗组 (N=29)	28 (96.6%)	7 (24.1%)
对照组 (N=30)	22 (73.3%)	6 (20.0%)
χ^2	6.149	0.147
P	0.026	0.701

Discussion

麻桂温痹颗粒方中寓有上中下痛风方之意，以分清实邪，除痹止痛为意，方中麻黄为君发散风寒，桂枝为臣，温通经脉，祛在上之风，青风藤既能祛风邪于上，又能胜湿邪余下，又可散寒通络止痛，防风解表发汗，祛内外之风，又能胜湿止痛，与方中另外二味药共奏祛风散寒，除湿止痛之功。

通过 12 周的临床观察，显示联合麻桂温痹颗粒治疗 RA 较西药常规治疗能够更好地缓解关节疼痛，减轻关节肿胀，降低疾病活动度，降低 ESR 炎症指标水平，改善中医证候积分。总体疗效评价不仅获得了更显著的中医证候疗效，ACR 缓解率也优于对照组。分析患者 IL-23、IL-17A 细胞因子治疗前后的变化，发现联合本方能够更好地抑制促炎因子的分泌，可能是

因为本方能够对 IL-23/IL17 炎症轴发挥调节作用，从而调节细胞免疫平衡。

Conclusion

麻桂温痹颗粒的临床疗效通过中西医不同方面，临床症状体征、指标、炎症因子不同层面，进行较为全面客观的比较。期为临床治疗类风湿关节炎提供更优化的治疗方案，并为本方进一步研究其作用机制提供良好的基础。

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Content Validity of Thai Version Screening Tool for Cervical Spine Instability

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ABSTRACT

Introduction: Cervical spine instability is a disorder in which the cervical spine cannot maintain its normal alignment under physiological loads to protect the blood vessels, nerves, and spinal cord from damage or irritation. The symptoms of cervical spine instability present include neck pain, dizziness, inability to prolong holding the head, and impaired movement. The more severe symptoms can cause cervical spondylolisthesis. Therefore, it is necessary to have an early screening tool for cervical spine instability to avoid complications and consequences. However, this screening tool is limited in its use by language for the Thai therapist who is a non-English speaker.

Objective: The primary objective of this study was to translate the 16 items of the cervical spine instability screening tool from English to the Thai language. The secondary objective was to test the content validity of the cervical spine instability screening tool Thai version.

Methods: The cervical spine instability screening tool was translated from the original English language into the Thai language by the process of cross-cultural adaptation. Then, the content validity was tested by using the index of Item-Objective Congruence (IOC) which was obtained by a group of experts. 5 independent experts with clinical experience more than 5 years in treating neck pain patients including 3 physiotherapists and 2 orthopedic surgeons.

Results: The IOC index rating by 5 independent experts reached an average of 0.90 (range 0.60-1.00).

Conclusion: The screening tool (Thai version) for patients with cervical instability has satisfactory content validity (average IOC = 0.90) for application in clinical practice.

Keywords: cervical spine instability; non-specific chronic neck pain; content validity; screening tool

Introduction

Neck pain is one of the most common musculoskeletal pains among the population, which can be suffered at any age [1]. The prevalence of neck pain ranged from 48.5% of people over their lifetime (range:

6.9% -54.2%) and 29.8% of people in the past six months (range 14.2%- 71%) reported by the previous prevalence studies [2]. Moreover, contributing factors for neck pain include poor posture, neck injuries, degenerative changes [3], working more than four hours

daily, stress, etc. [2]. Patients with neck pain usually suffer from symptoms such as arm pain/numbness, nerve root irritation, and spinal cord compression, depending on the location of the pathology. Therefore, limitation of daily activities and eventually a decline in their quality of life can occur due to neck pain. Current treatment of neck pain is mainly managed with supported treatment, including heat, cold, physical therapy modalities, and exercise to relieve pain. However, there may be a need for surgery in some cases. The literature reported that cervical degeneration was associated with cervical spine instability [5]. Acute or chronic injury from work induced fluid in the herniated disc (nucleus pulposus) to flow out and decreased disc height and make disc degeneration, annulus fibrous ruptures, disc herniated, and lead to cervical spine instability [6]. Cervical spine instability is a disorder in which the cervical spine cannot maintain its normal alignment under physiological loads to protect the blood vessels, nerves, and spinal cord from damage or irritation [7]. Neck pain, pain/numbness in the arm, dizziness, inability to hold head for long periods, hypermobility of movement, impaired movement, or cervical spondylolisthesis are all symptoms of cervical spine instability [8]. Therefore, early screening for cervical spine instability is important to avoid complications and consequences. According to the literature review, X-rays in full flexion and extension postures are most commonly used to diagnose cervical spine instability [10,11]. When the vertebral displacement is greater than 3.5 mm or the angle is greater than 11 degrees, the patient is judged to have cervical spine instability [7]. Furthermore, patient history is considered during the clinical diagnosis process [8]. A common question may not cover all symptoms of patients with cervical instability. However, Cook et al. (2005) found the symptoms to screen for cervical spine instability, which contained 16 questions covering the symptoms of cervical spine instability. However, this questionnaire is written in English and has not been translated into Thai. Due to language difficulties, this makes it difficult to use and may result in errors in the use of this screening form. As a result, the objectives of this study are to translate the cervical spine instability screening tool into Thai and test the content validity of a screening tool for evaluating Thai patients with cervical spine instability.

Methodology

The procedure was divided into two stages: 1) development of the Thai cervical instability screening tool and 2) testing content validity of the tool.

Development process of Thai cervical instability screening tool

Step 1: Forward translation

The original English version was translated into Thai by three native Thai speakers with advanced English skills. One of the translators is an orthopedic surgeon, while the others are physical therapists who are experts in musculoskeletal fields. All of the translators have at least five years of clinical experience. As a result of this process, three Thai language variations were created. (version 1: T1, T2, and T3).

Step 2: Compared and discussed/synthesis

The three translators discussed the three different versions (version 1: T1, T2, and T3) in detail for each item. After that, a new version of the screening tool was generated (T123). The best translated version should be reflective of Thai culture and acceptable to at least two translators. Thai language variants emerged as a result of this stage (version 2: T123).

Step 3: Backward translation

Three native English speakers who can read and understand Thai, there were a general native English speaker, a Thai physical therapist, and Thai orthopedic surgeon working abroad who had never seen the original version, translated the Thai version (version 2: T123) into the English language (version 3: BT1, BT2, and BT3).

Step 4: Comparison and Discussion

Version 3 (BT1, BT2, and BT3) and the original version were compared to consider the meaning of each item by two researchers. Both were native Thai speakers with excellent English skills. If it is discovered that a question has a different meaning from the original, it will be investigated and corrected by the translators immediately. The process was repeated until the questions had the same or similar meaning as the original, resulting in version 4: BT123.

Step 5: Expert committee review

The physicians who understand and are familiar with the assessment's content were involved in these steps, including three physical therapists who are experts in musculoskeletal fields and two orthopedic surgeons who have experience of at least 5 years of treating neck pain patients. They examined all 3 versions of screening tools (version 2, version 4, and original) together, then corrected and revised them. As a result of this procedure, version 5 was created (pre-final version).

Step 6: Pretesting

Thirty participants with non-specific neck pain tested the questions on the assessment form version 5 for comprehension before using them. The comments section from participants' feedback will be provided for additional ideas. After then, the participants were asked whether they understood the answers to the questions that matched the study team's answers or not. If more

than 20% of the participants (more than 6 persons per question) did not comprehend the question, the research team revised it to make it more understandable and dispatched specialists to respond to the issue. It didn't need to be changed if the experts agreed. As a result, the questions as mentioned earlier were put to the same test on the same people. (Of the 30 participants, 10 were chosen randomly).

Content validity assessment of Thai cervical instability screening tool

The content validity was reported by the index of item-objective congruence (IOC). It was rated by 5 independent experts who have experience in treating patients with neck pain for more than five years, consisting of 3 physical therapists who are experts in musculoskeletal fields and 2 orthopedic surgeons. By using the criteria for consideration, the experts evaluate and determine each item for its quantifiable regard to content measurement standards. The expert review's rating was as follows: consistent +1, non-conforming -1 and unclear 0. After reviewing and obtaining a rating score for each item from the experts, this information was used to figure out the index of item-objective congruence (IOC). If the IOC value was ≥ 0.50 , the questionnaire was measured on purpose or it can apply to that question.

Results

Characteristics of participants

Thirty patients with non-specific neck pain with more than 3 months of symptoms were included in this study. The duration of pain ranges from 3 months to 10 years and the average pain intensity was 5.29 (range from 3.2 to 8.2.) rated by VAS. The detailed characteristics of the patients in this study were shown in table 1.

Table 1 Characteristics of the participants

Demographic Characteristics	N (%)	Mean±SD	Range
Age (y)	30	45.33±6.57	35-55
Sex			
Male	11 (36.67%)		
Female	19 (63.33%)		
Pain duration (mo)		16.80±23.78	3-120
3 mo to 1 yr	23 (76.67%)		
> 1 yr	7 (23.33%)		
Education			
Primary school	1 (3.33%)		
High school	5 (16.67%)		
University	24 (80.00%)		
VAS (0-10)		5.29±1.57	3.2-8.2

Development process of Thai cervical instability screening tool

At the final stage of the translation process, all items in the questionnaire were modified. Therefore, the final version of Thai was developed, which is easier to use.

Content validity assessment of Thai cervical instability screening tool

For all 16 items, content validity for the Thai screening tool was reported by the mean IOC of 0.90 (range 0.60-1.00). The IOC mean values for each item of the screening tool was presented in table 2.

Table 2 The content validity of each item of the screening tool

No.	Detail	IOC
1	Feeling fatigue when in a prolonged static posture	0.8
2	Fatigue and difficult to hold head up	0.6
3	Better when supporting the head with a hand or using cervical collar	1
4	Frequent neck manipulation own self	1
5	Feeling of instability, shaking, or uncontrol	1
6	Increase episode of acute neck pain	0.8
7	Sharp pain with sudden movements	0.6
8	Head feels heavy	1
9	Neck feels stuck or locked with movement	1
10	Better in unloaded position such as lying down	1
11	Neck catching, clicking, or popping	1
12	History of neck disorder or trauma	1
13	Neck pain with minimal head or neck movement	0.8
14	Feeling stiff or tightness of neck muscles	1
15	Fear of movement	1
16	Temporary improvement with neck manipulation	0.8

Discussion

Individuals suffering from chronic neck pain may have cervical spine instability due to ligament degeneration of the cervical spine. Because of this instability, a neighboring nerve becomes irritated and may eventually develop spondylolisthesis. Therefore, people experiencing neck pain should be screened to prevent as well as rule out spondylolisthesis. The most popular and widely used method of diagnosis for cervical spine instability is an X-ray. However, it has several drawbacks, including high costs, radiation exposure, and the inability to do it on specific patients, such as pregnant women. In addition, in clinical practice, the patient's history is screened. However, it is board screening with no specific evaluation. Moreover, the Delphi technique study investigates cervical spine instability symptoms, including 16 items. But its psychometric properties are inconclusive. The screening tool has developed and designed to be completed by physical therapists and doctors. A score range of 0 (not associated with cervical spine instability) to 16 (strongly about cervical spine instability). The mean age of participants included in our study was 45.33±6.57 years, which was consistent with the studies of Cramer et al. [12], Lim et al. [13], and Uthaihpun et al. [14], with a mean age of between 49±16 years, 44±12 years, and 48.7±11.3 years, respectively. In addition, the present study was the first study of the cervical spine instability

screening tool in Thai patients with non-specific neck pain. Although no research was conducted to determine the psychometric property of cervical spine instability, previous studies have demonstrated a relationship between chronic neck pain and instability of the cervical spine. So instead, a study was conducted to determine the psychometric properties of neck pain using a screening test called the neck disability index, which was administered to patients with neck pain all over the world and cross-cultural adaptation into various languages. According to the study's findings, there were no floor or ceiling effects between the studies by Uthaikhup et al. [14], which looked at the content validity of the NDI-TH, and Lim et al. [13], which looked at the content validity of the simplified Chinese version of the Neck Disability Index. The current study found the content validity of the cervical spine instability screening tool, which showed good content validity (IOC = 0.90). The strength of this study is that it is the first Thai language version of the cervical spine instability screening tool which was translated by Beaton guidelines [15]. The study also provided evidence of Indexes of Item-Objective Congruence (IOC) of the Thai version of the cervical spine instability screening tool. Nevertheless, our study still had some limitations. Other psychometric property values such as reliability, construct validity, convergent validity, and cut-off score have not been studied, so future studies will explore these values of this screening for further clinical benefit.

Conclusion

The screening tool (Thai version) for patients with cervical instability has satisfactory content validity (average IOC = 0.90) for application in clinical practice.

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Correlation between the 2- and 6- minute Walk Test in Healthy Children Aged 7-10 Years

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ABSTRACT

Introduction: The 2- and 6-minute walk tests are used to assess walking ability, but the 2-minute walk test is not commonly known.

Objective: The purpose of the research was to investigate the correlation between the 2- and 6-minute walk tests in healthy children.

Methods: A Cross-sectional study was carried out on healthy children whose age ranged from 7 to 10 in the first through third grades of primary schools in Chiang Mai province, Northern Thailand. Each participant also completed a 2- and 6-minute walk test on two separate test days by a week. Pearson's correlation coefficient (r) was calculated for the relationship between 2- and 6-minute walk test.

Results: In 2- and 6-minute walk tests, the mean walking distance was 203.6 meter from the 2-minute walk test and 559.79-meter from 6-minute walk test. A significant positive correlation was found between 2- and 6-minute walk tests, $r = 0.99$, $p = 0.001$ of all participants.

Conclusion: These data suggest that the 2- and 6-minute walk tests in healthy children have fairly strong positive correlation. Our findings support the 2-minute walk test is a suitable alternative timed walking test for functional capacity testing in children.

Keywords: 2-minute walk test; 6-minute walk test; correlation; children

Introduction

Walking is the principal means by which most humans go from one location to another under their strength.[1] If people are to function autonomously in the group, they must be able to walk a significant distance—more than 300 meters, according to Shumway-Cook et al.[2] Timed walk tests have been used to evaluate ambulatory endurance since the inception of the 12-minute walk test in the 1970s.[3] The 6-minute walk test (6MWT) was suggested by the American Thoracic Society (ATS) for this purpose[4] and has been used with the pediatric population.[5] Nonetheless, the 2-minute walk test (2MWT) may be used as an alternative assessment. Both 2- and 6- minute walk tests were used to measure self-paced walking ability and functional capacity. However, the 2-minute walk test has a shorter distance than the 6-minute walk

test. Which is suitable for person with disability of walking.[6]

The current situation of respiratory infections causing children to be affected after infection that affects activities. Therefore, the 2MWT is another option for physical capacity evaluation. Aside from taking less time than the 6MWT, the 2MWT is rarely exceeded during incidental bursts of walking.[7] Furthermore, the distance walked in 2 minutes is substantially linked with the distance reached in 6 minutes ($r = 0.968$)[7], and the walking pace during the 6MWT is constant between 1 and 6 minutes.[8] However, some children who are willing to walk for 2 minutes continuously "give up" before 6 minutes.[7] Although 2MWT is suitable for children, correlation with 6MWT has not been reported in Thailand. The purpose of this research was to investigate the

correlation between the 2- and 6-minute walk tests in healthy children.

Methodology

A cross-sectional study was conducted on healthy children from the first through third grades of primary schools in Chiang Mai province. The participants were randomly chosen; each school's administration chose healthy pupils at random whose parents agreed to their child's involvement and provided informed consent. The sample size calculation was calculated from the program G-Power version 3.1, which references a sample population based on research by Bohannon et al.[7] By setting Power equal to 0.80, and alpha level equal to 0.05, found that there should be a total number of not less than 26 cases in each group (effect size=1.032). For this reason, a total of 30 participants collected data. And the children have the ability to walk more than 60 meters. On the other hand, children with underlying disorders, particularly cardiovascular, respiratory, or neuromuscular problems, a history of severe sickness within the two weeks prior to selection, a history of taking medication that could impair the walk test.[9] Conditions that have a major impact on walking capacity and an inability to gain informed consent were also excluded.

The record form included date of birth, underlying conditions, age, gender, weight and height. The data was collected between April and May 2022. The chosen children were questioned by researchers and evaluated by physicians to establish their eligibility as subjects. A stadiometer and digital weighing scale were used to measure height and weight in the standard position. Using a conventional tape, leg length was measured from the anterior superior iliac spine (ASIS) to the medial malleolus in the upright posture.[10]

Before any performance-based assessments were conducted, participants' basic demographics (age, gender, height, body mass, etc.) were assessed. The participant sits in a chair near the start of the walking course while receiving instructions from the training handbook. The examiner gave one demonstration, then each participant took an out-and-back practice round before taking one trial of the actual examination. Participants were instructed to wear comfortable clothing and proper shoes and to refrain from excessive exercise for at least two hours prior to the test. They were required to rest for at least 10 minutes in a chair at the starting line before beginning the test. During the waiting time, participants' respiration rates, pulse rates, and blood pressure were monitored, and they were asked to score their baseline fatigue or dyspnea using the Borg CR10 scale (0 = no exertion at all and 10 = maximal exertion).[10] Each participant also completed a 2- and 6-minute walk test on order with two separate test days by a week. The exams were held between 8.30 a.m. and 11.30 a.m. at a location designated on the grounds.

Two-minute walk test

The 2MWT was completed on a 50-foot (15.2-meter) out-and-back course. A stopwatch, measuring tapes, cones, marking tapes, a chair, record sheets, and a clipboard were used in the setup. Before the participant came, the examiner marked a 50-foot course with 10-foot intervals in a broad, flat, open area such as a hallway, with a cone at either end. A chair was strategically positioned near the start of the route. Participants were told to walk as quickly as they could until they were told to stop (without anyone walking with them). They were also urged not to worry if they needed to slow down or rest, but that if they did, they should resume walking as soon as they were ready. They were told after 1 minute, "you are doing great; you have 1-minute remaining." Participants came to a halt after 2 minutes of walking.[9] The examiner placed a piece of tape behind the participant's heel to note where the participant stopped and measured the distance from the tape to the last lap. Finally, the examiner recorded the distance on the record sheet. The test, including instructions and practice, took about 4 minutes to conduct.

Six-minute walk test

The 6MWTs were carried out in accordance with the American Thoracic Society (ATS) recommendations [11]. The technician who administered the 6MWTs was advised about the standard protocol and instructed to utilize it. A conventional 30-meter tape was used to measure the five 30-meter walking courses on long, level, straight, and hard ground. Every meter of the walking paths was labeled. The starting and turnaround points were also delineated. Each lap covered a total distance of 60 meters.

The test was timed for six minutes using a regular stopwatch. The technician utilized standard terms of encouragement during the testing to motivate the participants in the same way. When the six minutes were up, the individuals were instructed to stop walking and the technician recorded the total distance from the test. The same technician monitored the participants' respiratory rate, pulse rate, and blood pressure before asking them to rate their post-walk Borg CR-10 scale. Dizziness, chest discomfort, leg or thigh pain, and syncope were all reported as post-walk symptoms. The participants were applauded and given a drink of water before being sent back to their classes by walking. Ethical review and approval were approved by the Institutional Review Board of the Faculty of Medicine of Chiang Mai University (IRB number 511/2021).

Statistical analysis

The descriptive characteristic was used to represent participant characteristics and distance from walking tests. Shapiro-Wilk test was used to confirm normal distribution of data. Pearson's correlation (r)

was used to assess the correlation coefficient between 2- and 6- minute walk tests. All analyses were carried out in SPSS version 26 (significant at alpha 0.05).

Results

A total of 30 participants were investigated during the study; 50% were males (Table 1). The comparison of baseline characteristics has similarities between male and female ($p > 0.05$). The Borg scale was grade 0 for all participants prior to the assessment of the 2- and 6-minute walk tests. As shown in Table 1.

Table 1 Male and female characteristics (mean \pm SD)

	n=30	Male (n=15)	Female (n=15)
Age (year)	7.96 \pm 0.71	8 \pm 0.75	7.93 \pm 0.7
Weight (kg)	17.06 \pm 1.31	17.13 \pm 1.18	17 \pm 1.46
Height (m)	1.29 \pm 5.1	1.28 \pm 5.1	1.30 \pm 4.7
BMI (kg/m ²)	10.21 \pm 0.63	10.39 \pm 0.51	10.04 \pm 0.71
Leg length (cm)	64.64 \pm 1.17	64.3 \pm 1.14	64.99 \pm 1.13
2 MWD (m)	203.6 \pm 17.4	205.4 \pm 20.07	201.8 \pm 14.9
Borg-post	2(1,3)	2(1,3)	2(1,3)
6 MWD (m)	559.79 \pm 50.97	605.06 \pm 58.82	594.51 \pm 43.16
Borg-post	5(4,7)	5(4,6)	6(4,7)

Continuous variables (age, height, weight, leg length, and BMI-Body Mass Index) are given as mean values with their standard deviations (SD). Borg scale is present with median and interquartile range (IQR1,3).
 n: number of participants; MWD: minute walk distance; kg: kilogram; m: meter; cm: centimeter.

Table 2. Pearson correlation coefficients (r) between 2- and 6- minute walk tests in children

Pearson correlation (r)	2MWD	6MWD	age	weight	height	Leg length	Borg scale-post (2MWT)	Borg scale-post (6MWT)
2MWD (r) (p-value)	1	0.99 ^b 0.001*	0.90 ^b 0.001*	0.56 0.001*	0.40 0.02*	0.44 0.01*	0.02 0.88	-0.3 0.85
6MWD		1	0.89 ^a 0.001*	0.538 0.002*	0.38 0.03*	0.42 0.02*	0.02 0.90	-0.04 0.81
age			1	0.51 0.004*	0.28 0.12	0.33 0.06*	0.14 0.44	0.05 0.76
weight				1	0.56 0.001*	0.54 0.002*	0.20 0.27	0.17 0.35
height					1	0.92 ^b 0.001*	0.11 0.54	-0.08 0.66
Leg length						1	0.13 0.48	-0.49 0.79
Borg scale-post (2MWT)							1	0.10 0.59
Borg scale-post (6MWT)								1

*Indicates statistical significance ($p < 0.05$), ar=0.7-0.89, br=0.9-1.00, Minute walk distance (MWD).

The relationship between 2- and 6- minute walk distance (MWD)

There was a strong and significant positive correlation between 2MWD and 6MWD ($r = 0.99$, $p = 0.001$). Correlations calculated for the four variables and 2MWD, also showed the significance ($p < 0.05$). In 2MWD they were, $r = 0.56/0.01$ (weight), $r = 0.40/0.02$ (height), and $r = 0.44/0.01$ (leg length) respectively. As shown in Table 2.

Discussion

The primary finding of this study is that the 2MWT and 6MWT are highly correlated. A correlation is dominant in the relationship between the 2MWT and 6MWT ($r = 0.99$, $p = 0.001$). These results are

comparable with earlier research on the 2MWT and

6MWT, in previous study (2015-2018) ($r = 0.61$ – 1.00). [6, 8, 12] According to a general recommendation, $r > 0.75$ indicates good correlation, and $r > 0.90$ is suggested for clinical choices.[9]

Despite the 2MWT and 6MWT's strong relationship, there was a statistically significant increase in walking distance from the children group (203.6 \pm 17.49 m and 559.79 \pm 50.97 m in the 2MWT and 6MWT, respectively). The variation was also observed in children's groups, but most of it was not statistically significant. The results could be explained by small sample size and age range is not wide enough.

For healthy youngsters, the 6MWT is recognized as a valid and reliable functional walk test.[10] It is

feasible and useful, simple to implement, affordable, safe, and well tolerated by young children. In the previous study, the 6MWT has several factors involved in the result for interpretation including age, gender, weight, and height. Similarly, the 2MWT is most likely dependent on 6MWT.[9] In this study, the 2MWT is positively related to BMI, leg length, and Borg scale. According to Bohannon (2018), provide normative values for the 2MWT for children and adolescents. Gender and age have a significant interaction with 2MWT from regression analysis ($F=2.27$, $p=0.05$). Correlation analysis revealed that age, age-squared, height, and mass were all moderately linked with 2MWT distance ($r=0.30$ to 0.53 , $p<0.01$).[7] Bohannon in 2015 described the norms of the 2MWT for healthy adults. The results of distance covered are correlated with participant's body mass index ($r=0.573$, $p<0.05$).[12]

However, there are several studies suggesting that 2MWT could assess ability of walking in children with neuromuscular disorder (eg. Myotonic dystrophy type 1, Becker muscular dystrophy, Duchenne muscular dystrophy, spinobulbar muscular atrophy, and facioscapulohumeral).[8, 9] The pathology of the disease is a result of a significant in decrease walking distance, which affects the interpretation of 6 MWT. As a result, the 2 MWT is appropriate for assessing children with functional impairments.[9]

Due to the current epidemic of respiratory disease, the functional ability assessment is important to predict the functional level and ability of daily life activity- especially walking. For this reason, the 2MWT is another available alternative assessment and suitable for young children. They have limited endurance and tolerance for long-distance walking, especially in children with long covid-19 symptoms.[13] The limitation of this study was that it was only studied in healthy subjects. Therefore, data cannot be inferred in children with underlying medical conditions. Although the 2MWT takes less time and is more likely to be completed by boys and girls than the 6MWT [7], its utility has been restricted to far due to a lack of norms in Thailand. Therefore, further studies should be done to determine the normative values in children.

Conclusion

The 2MWT and 6MWT have a good relationship in children. In which to measure the 2MWT, individual characteristics should be considered. A walking capacity resulted in an increase in absolute measuring inaccuracy. When evaluating 2MWT and 6MWT data in individual patients in the clinic, practitioners must be mindful of the challenges related to absolute functional capability.

Competing Interests

The authors declare that there is no conflict of interest.

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Author contributions

WC takes full responsibility for the integrity of the data and the accuracy of the data analysis. WC, RM, and SD had access to, and verified, the underlying data. WC, and RM critically evaluated all stages of the research process. All authors designed the study, contributed with conceptual design, analyses, and interpretation of the results, also contributed with drafting the article and critically revising it for important intellectual content, and gave final approval for the version to be submitted.

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A Pilot Study of Chemical Composition and Major Compounds of Conservation Plant: A Case Study of Ma-Kwan from Doi Phu Kha, Nan Province

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ABSTRACT

Introduction: The plant genetic conservation project under the royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn in response to the royal initiative by Mae Fah Luang University Botanical Garden has been a continuous survey and study on the utilization of indigenous plants. The results found that 391 types of Lua people and residents of Doi Phu Kha, Nan Province, categorized as food plants, medicines, housing, equipment and utensils. plants used in ceremonies and found an important spice is Ma-kwan (*Zanthoxylum limonella*).

Objective: The aim of this pilot to study the chemical composition of conservation plant and create a database of chemical composition of Ma-kwan that found in Doi Phu Kha, Nan Province.

Methods: The fresh Ma-kwan were dried, pericarps and seeds were separated and coarsely grinded then the essential oils were collected by water distillation method. The essential oils from pericarp and seed's part were analyzed for their chemical composition using Gas Chromatography-Mass Spectrometry (GC-MS).

Results: 35 compounds were found in essential oil; Piperitenol and p-Cymene were the most major compounds from pericarp and seed respectively. Moreover, essential oils obtained from the pericarp and seed have 16 identical compounds.

Conclusion: From the results of this study, the quantity and chemical composition of the essential oils from the fresh fruits will be recorded for comparison with fresh or dried fruits from different sources. They also know the major compounds found in the essential oils of fresh Ma-kwan found in Doi Phu Kha, Nan Province.

Keywords: Ma-kwan; *Zanthoxylum limonella*; Chemical composition; Essential oils

Introduction

Thailand has abundant natural resources and high biodiversity, especially in the northern region or the area known as the former Lanna Kingdom but after high economic growth and rapid population growth, there have been developments in various fields that have resulted in a change in the old way of life of the people in the area. However, in the northern area of Thailand, it is also the habitat of various ethnic groups that have the wisdom to use resources in the area. Plants used as a medicine as food and others, resulting in these plants being widely used. But, the utilization of some plants in the local area has not been scientifically studied to support it. The study of the chemical composition and major compounds of local plants is therefore an important study in the conservation of local knowledge

in terms of plant utilization and can also be disseminated to interested people or related groups to develop applications in parallel with current knowledge. Because of the plant genetic conservation project due to the royal initiative Her Royal Highness Princess Maha Chakri Sirindhorn Her Royal Highness Princess Maha Chakri Sirindhorn In response to the royal initiative by Mae Fah Luang University. There has been a continuous survey and study on the utilization of indigenous plants. The results of the study found that a study of the indigenous botany of Lua people and residents of Doi Phu Kha, Nan Province found a total of 391 types of plants, categorized as food plants, medicines, housing, equipment and utensils. plants used in ceremonies and found the important spice is Ma-kwan (*Zanthoxylum limonella*), which is widely used in cooking in the

northern region of Thailand. It is commonly used as a spice for flavoring food. It has a spicy flavor, while the bark and fruit can be used as a carminative. and as a sedative or pain reliever in India, coconut oil is commonly used as an anti-inflammatory in the name of “Mullilam oil”, therefore, Ma-kwan samples were collected from Doi Phu Kha, Nan province, to extract volatile oil and analyze its chemical composition. The aim of this pilot to study the chemical composition of conservation plant and create a database of chemical composition of Ma-kwan that found in Doi Phu Kha, Nan Province. Ma-kwan has a scientific name that *Zanthoxylum limonella* is in the family Rutaceae is a perennial plant that the northern people used as fruit and dried seeds for cooking; including used to make herbal medicine. Because, the dried fruit has a strong smell and has a spicy flavor helps to deodorize the fishy smell and enhance the flavor of food, it is also popular to use the dried fruit to extract essential oils for massage, used as a cosmetic ingredient or use anti-mosquito spray [1]. Botanical characteristics of Ma-kwan, it is a medium to large deciduous tree, 12-20 m tall, bark white, with conical spikes at the end, straight or slightly curved, growing along the trunk, branches and petioles 10-28 leaflets, may or may not have leaflets at the ends. Petiole short 0.5-1.0 cm, leaf width 4-5 cm, length 10-14 cm, oval, ovate or oblong, base ovate, margin smooth or wavy. The tip of the leaf is slender and pointed. The flowers are panicle-like panicles at the apex or axillary leaves. Inflorescences are 10-21 cm long, peduncles are long, small, greenish-white flowers are clustered at the end of the bouquet. The pistil and the male flower are on different plants, 4 secondary petals, 4 petals arranged alternately with 4 stamens, 1 pistil above the stamens, inside there is 1 cavity. Fruit round shape, light green fruit, 0.5-0.7 cm in diameter. The taste is very spicy when the skin is brown and crack open to see the black seeds into it. Flowering - fruiting during March – April [2]. Ma-kwan is found in the north of Thailand, naturally found in dry evergreen forests or evergreen forest in addition, it was also found that farmers prefer to plant interspersed with wild garden plants, which is planted together with other crops to increase their income. Sources of quality Ma-kwan such as Pha Chang Noi Sub-district, Pong District, Phayao Province, Doi Hang Subdistrict, Mueang District, Chiang Rai Province, Pa Pae Sub-District, Mae Taeng District, Chiang Mai Province, Doi Phu Kha, Pua District, Nan Province [2]. Ma-kwan can grow in areas with an altitude of 800 - 1,000 meters above sea level in cool climates and high humidity. It thrives in outdoor conditions, does not require much water likes soil that drains well, so it should be planted along the hillside or steep areas. Generally, Ma-kwan is used by using mature seeds and it is the fresh seeds that come out of the new husks, not dry. Ma-kwan will begin to produce at the age of 3 - 5 years. There are heavy varieties and light varieties, light varieties will begin to bloom in July and harvested in

October - November, the heavy varieties will begin to bloom in August and harvested in November-December, 3 - 5 years of age has yield approximately 1 - 5 kg dry per plant, 6 - 10 years old has yield 10 - 15 kg dry per plant, 11 - 15 years old has yield 30 - 35 kg dry per plant and 21 - 25 years old yields up to 50 kg dry per plant [1]. Because Ma-kwan is a spice plant used in local food. Most of the Ma-kwan market is in the northern area. The price of Ma-kwan that farmers can sell in the range of 50 - 70 baht per kg dry. In addition to farmers selling their products, then it is also processed into products of soup, stew, chili paste, etc. The parts used in cooking are young leaves and fruits, leaves and young leaves can be eaten as fresh vegetables. The fruit is a popular spice “Larb chili paste” seasoning has a spicy flavor that helps to improve the taste of food. Ma-kwan has folk wisdom that it is used as a side dish for meat-rich dishes because it can digest meat. In the south, it is popular to mix mackerel in spices such as pumpkin curry, eel curry, etc., which gives the curry a hot and spicy flavor and smells good. Ma-kwan has used as drug properties in traditional medicine; the roots and wood are used as intestinal carminatives, reduces dizzy, reduces blood pressure and it is a menstrual blood pill for women but not for pregnant women. Seeds can extract essential oils for treat dizziness, nourish blood, nourish the heart, increase blood circulation. Using in Chinese medicine textbooks to cure stomachache, flatulence, indigestion, vomiting, diarrhea, Ma-kwan seed extract, it has an anti-inflammatory effect caused by the poisoning of Formalin and Carrageenin and can suspend inflammation on the skin when applied with oil extracted from the Ma-kwan has the effect of suicide intestinal parasites [3]. From the study of chemotaxonomy of plants found that extracts from plants in the family Rutaceae have anti-inflammatory effects, especially citrus plants (Citrus) [4]. The main chemical composition of the skin is monoterpene, containing α -terpinene, α -terpineol, terpinen-4-ol, terpineol, α -pinene, sabinene and limonene, which has the highest concentration as seen in the effects of anti-inflammatory [5] by mechanism can inhibit the production of nitric oxide, prostaglandin E2 and TNF- α [6]. In addition, studies on the bioactivity of limonene showed that at a concentration of 50 μ g/ml suppressed the expression of limonene. The cyclooxygenase-2 (COX-2) gene was not significantly different from the 20 μ M concentration of celecoxib by the induction of cyclooxygenase-2 (COX-2) genes. Lipopolysaccharides (LPS) in RAW 264.7 macrophages [7] and 0.04% concentrations inhibited prostaglandin E2, equivalent to that of 20 μ M dexamethasone inhibited pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) compared to levels of TNF- α , IL-1 β and IL-6 production in RAW 264.7 macrophages by induction of LPS and suppressing iNOS Protein and COX-2 Protein expression [8]. In vitro study found that extracts from the Ma-kwan fruit which is obtained by water

distillation has an inhibitory effect Cyclooxygenase-1 (70.4%), cyclooxygenase-2 (88.9%) and 5-lipoxygenase (7.57%) reported in recent studies have shown the bioavailability of extract to inhibit the neurotransmitter mediator of inflammation [9].

Methodology

Raw materials and preparation

The Ma-kwan fruits used in this study came from Doi Phu Kha in Nan province, harvested in October 2021. The fresh fruits were dried by the solar drying for 6 hours. Total weight of raw materials fruits in this study, when dried, was 30.88 kg, divided into pericarps weight 17.78 kg, seeds weight 13.10 kg. The pericarps and dried seeds were then separated and coarsely ground with a grinder for 20 seconds/kg, their weight and appearance were recorded.

Distillation of essential oils

The essential oil from the pericarps and seeds were extracted by water distillation using 150 liters of water and distilled for 6 hours in Clevenger apparatus at 60 Celsius degrees. Recorded the yellow oil content and appearance.

Chemical composition analysis

The essential oils obtained from the pericarps and dried seeds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with column TG-5 slims. Helium gas was carrier with flow rate 1.2 ml/min. The initial temperature of column oven was programed 120°C and then heated to 280°C with a rate of 8°C/min and kept constant at 280°C for 15 minutes. The mass spectrum was recorded. Chemical compositions were analyzed by Science and Technology Service Center, Faculty of Science, Chiang Mai University (STSC-CMU).

Results

The total weight of raw materials in this study, was 30.88 kg, divided into dried pericarps weight 17.78 kg and dried seeds weight 13.10 kg. Essential oil was extracted from the pericarps of 143 g (0.8% yield) and 66 g (0.5% yield) of the seeds.

35 compounds were found in essential oil, essential oils obtained from the pericarps and seeds have 16 identical compounds and 11 compounds from both parts, the chemical composition could not be identified. Piperitenol (30.6% yield), 3-Cyclohexene-1-methanol, alpha, alpha, 4-trimethyl-, acetate, (1R) (14.8% yield), p-Cymene (6.8% yield), Linalool (6.7% yield) and Limonene (4.8% yield) were major compounds from the pericarps (Figure 1) and p-Cymene (20% yield), Bicyclohexane, 4-methylene-1-(1-methylethyl), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R) (16% yield), Limonene (6.5% yield) and Linalool (4.6% yield) were major compounds from

the seeds (Figure 2).

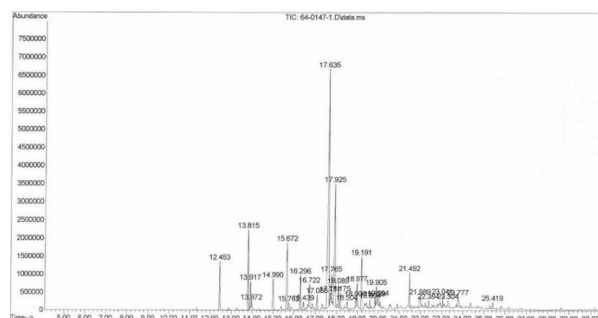


Figure 1 GC chromatogram of the pericarps

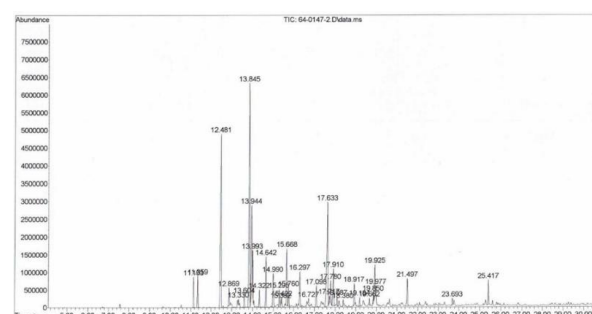


Figure 2 GC chromatogram of the seeds

Discussion

The chemical composition of this study was found to be similar in number of compounds to the study of Chemical compositions and pharmacological effects of essential oil from the fruit of *Zanthoxylum limonella*, evaluation of the oil composition was achieved by GC/MS analysis; Limonene, terpineol and sabinene were found to be the major components [10]. At the time of this study, the chemical composition of the compounds was found, but with different quantities. Because, this study found that Piperitenol, 3-Cyclohexene-1-methanol, alpha, alpha, 4-trimethyl-, acetate, (1R) and p-Cymene were major compounds from the pericarps and p-Cymene, Bicyclohexane, 4-methylene-1-(1-methylethyl), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R) and Limonene were major compounds from the seeds. Also consistent with the study of characterization of the essential oil and fatty oil from Ma-kwan fruit (*Zanthoxylum rhetsa*; *Zanthoxylum limonella*), the 24 compounds were identified in the essential oil, including monoterpenes, oxygenated monoterpenes, sesquiterpenes, hydrocarbon and ketones group; the major compounds from essential oil of Ma-kwan fruit were α -phellandrene, terpineol, sabinene and α -pinene [5]. From the above comparative studies, it can be concluded that the chemical composition of the essential oils of the Ma-kwan fruit is mainly Terpenes, but the difference may be due to the type of raw material, source of raw material and different in drying methods affect to result in different

chemical compositions and quantities as shown in this study above.

Conclusion

From the results of this study, the quantity and chemical composition of the essential oils from the fresh fruits will be recorded for comparison with fresh or dried fruits from different sources. They also know the major compounds found in the essential oils of fresh Ma-kwan found in Doi Phu Kha, Nan Province.

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Correlation between Perceived Severity of COVID-19 and Self-Protective Behavior during COVID-19 Pandemic in Chiang Rai Smokers

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ABSTRACT

Introduction: The COVID-19 disease affects among economy, society, and health in population. Smoking could increase chance of contracting COVID-19 and increase the severity level of COVID-19 symptom. No study about the impact of COVID-19, perceived severity of COVID-19 and smoking behavior in smoking population during COVID-19 pandemic in Chiang Rai.

Objective: There are three aims of this study which are to study smoking behavior, to study impact of the Covid-19 pandemic situation in Chiang Rai smokers and to study the correlation between perceived severity of COVID-19 and self-protection behavior during the COVID-19 pandemic in Chiang Rai smokers.

Methods: The smokers have smoke at least 3 years, aged 18-60 years old and stayed in Chiang Rai were recruited in this study. A self-administered anonymous questionnaire was used to collect information via google form program. The questionnaires comprising 5 parts consisted of sociodemographic data, smoking behavior, impact of smoker on the covid-19 situation, perceived severity of covid-19 and protective behavior from covid-19.

Results: 215 smokes were recruited in this study. The most participants are 200 (93.0%) males, mean aged 33.03 (± 12.38) years old. Mean duration of smoking is 15.11 (± 11.98). Smoking behavior, they usually buy cigarettes from retail stores, 1 time a week, each time bought a pack of cigarettes, an average cost of 61-80 bath a time and average monthly cost of 101-500 baht. COVID-19 impact to income and expense of smokers. The protection yourself by wearing a mask, wash your hands with soap or alcohol gel and keeping distance in Chiang Rai did not affect the smoking behavior and the purchase of cigarettes among the smoking population in Chiang Rai. The correlation between perceived severity of COVID-19 and self-protective behavior from COVID-19 is 0.166 (p-value = 0.015).

Conclusion: There is not impact of the COVID-19 pandemic on smoking and the vigilant measures against the spread of covid-19 excepted economy. Poor correlation between perceived severity of COVID-19 and self-protective behavior from COVID-19.

Keywords: correlation; Covid-19; perceived severity; protective behavior; Smoking

Introduction

Cigarettes are one of the biggest public health threats in the world by killing more than 8 million people per year [1]. 72,656 Thais died from smoking [2]. In Chiang Rai, it was found that there is a population aged 15 years and over, smoking 57,045 people from the population that has been registered 664,767 people [3].

Smoking is also responsible for the most common causes of death, including cardiovascular disease, emphysema and lung cancer, respectively [4]. Each smoker has a different risk of developing diseases based

on genetics, length of time smoked, amount smoked, and smoking habits and other risk factors.

Cigarette consumption behavior throughout Thailand in 2017 found that there were 10.7 million people aged 15 years and over, with the average age of first smoking being 18.1 years old [5]. Most cigarettes smoke a larger proportion of packs than cigarettes and the average monthly cost of buying cigarettes is 546 baht [5].

In the epidemic situation of the corona virus Globally, there are more than 239 million infected people and more than 4.8 million cumulative deaths

from Covid-19, 1.7 million in Thailand and 18205 respectively [3]. The economic, social, health and public health impacts of dealing with emerging diseases in urban lockdown. Chiang Rai Province was affected in two main areas, comprising effects on border trade and tourism [6]. As a result, the income of the population in Chiang Rai decreased [7].

Smoking increases the chances of contracting COVID-19 because while smoking increases the respiratory rate. This results in a change in nasal breathing to mouth breathing. The mucus becomes dry and sticky. Cilia's movements slowed down. As a result, the filtration efficiency of the respiratory system is reduced [8]. The more smoking that involves repeatedly touching the face with your hands, the easier it is for the virus to enter the body.

From previous studies on smoking in COVID-19 patients, it was concluded that both current and former smokers smoked cigarettes [9]. Smokers with infected COVID-19, there could be a higher percentage of the severity rating. The patient could have a chance to use a ventilator and was admitted to the ICU [10].

No previous research study about the smoking behavior, impact of the Covid-19 pandemic situation and correlation between perceived severity of COVID-19 and self-protection behavior during the COVID-19 pandemic in Chiang Rai smokers

The aims of this study are to study smoking behavior, to study impact of the Covid-19 pandemic situation in Chiang Rai smokers and to study the correlation between perceived severity of COVID-19 and self-protection behavior during the COVID-19 pandemic in Chiang Rai smokers.

Methodology

Study Design and Participants

This cross-sectional study was conducted in smoking population, Chiang Rai province The search was restricted to studies between November 2021 to February 2022. Eligibility criteria were: 1) live in Chiang Rai; 2) aged 18 to 60 years old; 3) a current smoker; 4) smoking at least 3 years. The people who were unable to read Thai were excluded in this study. The study protocol was approved by the Human Ethics Committee of the Mae Fah Luang university (Protocol No: EC 21184-25). All participants signed consent form before they did the online questionnaire. Only the researchers could access the data for data prevention. The snowball sampling technique was used to recruit participation.

Data Collection and Questionnaire Development

A self-administered anonymous questionnaire was used to collect information from 215 people via google form program. The questionnaire was created according to the objective, key word of the study and the literature review. The content validity of the questionnaire was

examined by 3 experts consisting of tobacco, covid-19, and cardiopulmonary experts that found the content consistency index (IOC) = 0.67. Then the questionnaire was tested by a current smoker in Chiang Rai for the use of appropriate language, Test-retest reliability is 0.969, p-value < 0.001. The questionnaires comprised 5 parts sociodemographic data 13 items, smoking behavior 10 items, impact of smoker on the covid-19 situation 9 items, perceived severity of covid-19 10 items and protective behavior from covid-19 10 items. Totally of the questionnaire are 52 questions.

Perceived Severity of COVID-1. Totally questions are 10. (Known = 1 point, Unknown = 0 point). Therefore, fully score are 10 points.

Behaviors to protect yourself from COVID-19 infection in smokers are totally 10 questions. Each question is 2 points. (0 = Not implemented at all, 1 = Practice sometimes, 2 = Regular practice). Totally full score are 20 points.

Statistical Analysis

SPSS version 23 was used to statistically analyze the data. Descriptive statistics for continuous variables as means \pm standard deviation, while categorical data were reported by frequency and percentage. The Kolmogorov Smirnov test was used to test normal distribution. The test-retest reliability and correlation between perceived severity of COVID-19 and self-protective behavior from COVID-19 was statistically analyzed by Spearman rank correlation coefficient. It was classified by Chan YH, 2003 (1 Perfect correlation, 0.8-0.9 Very Strong correlation, 0.6-0.7 Moderate correlation, 0.3-0.5 Fair correlation, 0.1-0.2 Poor correlation and 0 No correlation) [11].

Results

215 smokers were recruited in this study. They were able to complete the questionnaire within 15-20 minutes. The most participants are 200 (93.0%) males, mean aged 33.03 (\pm 12.38) years old, mean weight 70.28 (\pm 15.88) kg. and mean height 169.90 (\pm 6.76) cm. Most participants are single, their level of education is senior high school and no underlying disease. Most occupations are general employees, their monthly expenditure was between 10,001 and 15,000 THB. The mean age to start smoking is 17.38 (\pm 3.49), the mean duration of smoking is 15.11 (\pm 11.98). Most of which are the reason for choosing to smoke because they want to try and have a history of having completed 2 vaccinations. Demographic data of all participants was shown in table 1.

Table 1 Demographic data (n=215)

Demographic data	Number (%)
Gender	
Male	200 (93.0)
Female	15 (7.0)
Age (years), mean (±SD)	33.03 (±12.38)
Weight (kilograms), mean (±SD)	70.28 (±15.88)
Height (centimeters), mean (±SD)	169.90 (±6.76)
BMI (kg/m ²), mean (±SD)	24.28 (±4.92)
Status	
Single	147 (68.4)
Married	62 (28.8)
Widowed/Divorced	6 (2.8)
High level of education	
Elementary school	28 (13.0)
Secondary school/Diploma	105 (48.8)
Bachelor's degree	78 (36.3)
Postgraduate	4 (1.9)
Underlying disease	
Yes	52 (24.2)
No	163 (75.8)
Occupation	
Student	62 (28.8)
Self-employed/ Trading	18 (8.4)
Government official	26 (12.1)
Private company employees	12 (5.6)
Agriculturist	9 (4.2)
General Contractor/Temporary Worker	81 (37.7)
No occupation	7 (3.3)
Monthly income (baht)	
No income	15 (7.0)
Less than 5,000	23 (10.7)
5000-10,000	56 (26.0)
10,001-15,000	74 (34.4)
15,001-20,000	22 (10.2)
More than 20,000	25 (11.6)
The age of start smoking (year), mean(±SD)	17.38 (±3.49)
The duration of smoking (year), mean(±SD)	15.11 (±11.98)
The reason for starting to smoke	
For relaxing	50 (23.3)
Want to try	141 (65.6)
For looking stylish and cool	19 (8.8)
For socializing	94 (43.7)
Have stress/anxiety	92 (43.3)
To show maturity	6 (2.8)
To make more confident	15 (7.0)
According to their favorite celebrity or person as a family member	3 (1.4)
	8 (3.7)
Vaccinated against COVID-19	
Not get vaccinated	10 (4.7)
1 dose	29 (13.5)
2 doses	141 (65.6)
3 doses	35 (16.3)

time bought a pack of cigarettes, an average cost of 61-80 baht a time and average monthly cost of 101-500 baht, respectively. Purchasing behaviors during COVID-19 (n=215) is shown in table 3.

Table 2 Smoking behavior (n=215)

Smoking behavior	Number (%)
Type of cigarettes	
LM	115 (53.5)
Self-rolled cigarettes (powder cigarettes/tobacco rolls)	56 (26.0)
Marlboro	56 (26.0)
Krong Thip	27 (12.6)
Sai-fon	9 (4.2)
Garam	12 (5.6)
Cigar	9 (4.2)
Pipe	6 (2.8)
Electronic cigarette	82 (38.1)
Other (iSCORE, Mond, Wonder, Atlanta, SMS, Camel)	22 (10.2)
The number of cigarettes per day (Roll)	
10 or less than 10	127 (59.1)
11-20 rolls	48 (22.3)
21-30 rolls	4 (1.9)
More than 31 rolls	2 (0.9)
No (in case of smoking only e-cigarettes)	34 (15.8)
The most smoking time	
After waking up	22 (10.2)
After eating	84 (39.1)
After exercise	4 (1.9)
Free time	88 (40.9)
While going to the bathroom	11 (5.1)
Before going to bed	6 (2.8)
The most frequency smoking place	
Bathroom	
School	32 (14.9)
Residence/House/Condo	2 (0.9)
Public places (e.g. parks, shopping centers, street corners, stadiums)	49 (22.8)
At social events/entertainment venues	3 (1.4)
Smoke when there is a smoking place	26 (12.1)
No place to choose, depending on the occasion.	46 (21.4)
Other, specify	51 (23.7)
	6 (2.8)
The most person who smoking with is	
Single smoker	160 (74.4)
Smoking with 1 other person	26 (21.1)
Smoking with more than 1 person	29 (13.5)

Smoking behavior is shown in table 2. This study showed LM was the most type of cigarette. The number of cigarettes smoked per day is about 10 or less than 10. Most smokers are smoking alone. They usually buy cigarettes from retail stores, 1 time a week, each

Table 3 Purchasing behaviors during COVID-19 (n=215)

Purchasing behaviors	Number (%)
In 30 days where did you buy cigarettes?	
Retailers/Stalls	147 (68.4)
Let others buy instead	23 (10.7)
Department stores/ Supermarkets	73 (34.0)
Website	26 (12.1)
Restaurant	10 (4.7)
The most frequency of buying cigarettes	
1 time/week	92 (42.8)
2 time/weeks	40 (18.6)
More than 2 time/weeks	83 (38.6)
Average cost of each cigarette purchase	
Not more than 40 baht/time	38 (17.7)
41-60 baht/time	25 (11.6)
61-80 baht/time	96 (44.7)
More than 80 baht/time	56 (26.0)
The cost of buy cigarette per month	
Less than 100 baht	25 (11.6)
101-500 baht	98 (45.6)
501-1,500 baht	64 (29.8)
1,500-2,500 baht	19 (8.8)
More than 2,501 baht	9 (4.2)

Impact of the COVID-19 pandemic on smoking and the vigilant measures against the spread of covid-19 don't affect to smoking in Chiang Rai smokers as shown in Table 4.

The score of Perceived Severity of COVID-19 is Median = 9. Interquartile range (IQR) = 7, 25th percentile (Q1) = 3, 75th percentile (Q3) = 10. There is lowest score is 0 and highest score is 10 as shown in table 5.

The results of behaviors to protect themselves from infection with COVID-19 showed mean of behaviors to protect yourself from COVID-19 infection in smokers is 15.1 (\pm 3.3), lowest score is 3 and highest score is 20, respectively as shown in table 6.

Table 4 Impact of the COVID-19 pandemic on smoking (n=215)

Impacts from the Covid-19	Number (%)
Have you ever been infected with COVID-19?	
Never	209 (97.2)
Ever	6 (2.8)
Have you ever been quarantined for 14 days because of the risk of contracting COVID-19?	
Never	164 (76.3)
Ever	51 (23.7)
The spread of COVID-19. How does it affect your income?	
Unchanged	60 (27.9)
Decrease	21 (9.8)
Increase	134 (62.3)
The spread of COVID-19. How does it affect your expenses?	
Unchanged	64 (29.8)
Decrease	71 (33.0)
Increase	59 (27.4)
No income	21 (9.8)
The spread of COVID-19. How does it affect your purchase of cigarettes?	
Does not affect the purchase of cigarettes	187 (87.0)
Easier to buy	3 (1.4)
Harder to buy	25 (11.6)
Measures to prevent infection with COVID-19 with a mask How does it affect your smoking?	
Smoking the same/No effect on smoking	159 (74.0)
Smoke less	50 (23.3)
Smoke more	6 (2.8)
Measures to prevent infection with COVID-19 with social distancing How does it affect your smoking?	
Smoking the same/No effect on smoking	163 (75.8)
Smoke less	47 (21.9)
Smoke more	5 (2.3)
Measures to prevent infection with COVID-19 by washing hands How does it affect your smoking?	
Smoking the same/No effect on smoking	183 (80.5)
Smoke less	36 (16.7)
Smoke more	6 (2.8)

Table 5 Perceived Severity of COVID-19 (n=215)

Perceived Severity of COVID-19	Known (%)	Unknown (%)
1 Did you know that COVID-19 worsen lung performance	188 (87.4)	27 (12.6)
2. Did you know that COVID-19 worsen lung performance as well as smoking	171 (79.5)	44 (20.5)
3. Did you know that Covid-19 patients with a history of smoking have more severe symptoms than non-smokers	145 (67.4)	70 (32.6)
4. Did you know that Smokers are at risk of contracting COVID-19 higher than non-smokers	125 (58.1)	90 (41.9)
5. Did you know that Covid-19 patients with a long history of smoking had more severe symptoms than those who started smoking	127 (59.1)	88 (40.9)
6. Did you know that Covid-19 patients with a history of heavy smoking had more severe symptoms than those who smoked low amounts	140 (65.1)	75 (34.9)
7. Do you know that COVID patients who have a history of smoking and have congenital disease? More severe symptoms than smokers without underlying medical conditions	144 (67.0)	71 (33.0)
8. Did you know that Covid-19 patients with a history of smoking had a higher mortality rate than those without a history of smoking	134 (62.3)	81 (37.7)
9. Did you know that secondhand smoke and the third hand affects the people around you, if infected with COVID-19 may make symptoms more severe	150 (69.8)	65 (30.2)
10. Did you know that Smoking and nicotine from cigarettes are among the strongest risk factors in infection with the COVID-19	121 (56.3)	94 (43.7)

Table 6 Behaviors to protect yourself from COVID-19 infection in smokers (n=215)

Behaviors to protect yourself from COVID-19	Regular	Sometimes	Not implemented at all
1. How often do you wear a mask every time you leave the house?	201 (93.5)	11 (5.1)	3 (1.4)
2. How often do you order food to come home to eat?	72 (33.5)	117 (54.4)	26 (12.2)
3. How often do you wash your hands with soap/alcohol gel when you come in contact with things and places outside the home?	151 (70.2)	63 (29.3)	1 (0.5)
4. How often do you keep a distance of 1-2 meters when you need to meet and talk to other people?	119 (55.3)	91 (42.3)	5 (2.3)
5. How often do you avoid doing activities in crowded places?	104 (48.4)	102 (47.4)	9 (4.2)
6. How often do you refrain from leaving the house to reduce the transmission of the virus?	80 (37.2)	121 (56.3)	4 (6.5)
7. How often do you not share things with other people?	125 (58.1)	75 (34.9)	15 (7.0)
8. How often do you not travel in and out of risky areas according to the provincial governor's announcement?	124 (57.7)	75 (34.9)	16 (7.4)
9. When you have a fever, cough, sore throat, see a doctor immediately.	92 (42.8)	98 (45.6)	25 (11.6)
10. You follow the news of the COVID-19 situation via television/internet/radio	155 (72.1)	55 (25.6)	5 (2.3)

Poor correlation between perceived severity of COVID-19 and self-protective behavior from COVID-19 is 0.166 (p-value = 0.015).

Discussion

Three aims of this study were to study smoking behavior, to study impact of the Covid-19 pandemic situation in Chiang Rai smokers and to study the correlation between perceived severity of COVID-19 and self-protection behavior during the COVID-19 pandemic in Chiang Rai smokers.

4.1 Smoking behavior

In the situation of the pandemic of COVID-19 Most of the reasons for smoking is wanting to try, followed by socializing. Follow up with friends, friends invite you to smoke, and have stress or anxiety. Make the most of your free time smoking. Without choosing a place depending on the occasion Most of them smoke alone. The number of cigarettes smoked per day was mostly about 10 roll or less and cigarettes were purchased from retail stores or stalls. Age at the start of smoking it was in the mean age range of 17 years, consistent with previous studies. As similar as the smoking behavior of 384 people in Surat Thani province [12], it was found that the average age of smoking for the first time of regular smokers was 17.8 years the reason why started smoking. Desire to try smoking accounted for 36.46%, and the number of cigarettes smoked less than 10 cigarettes per day, representing 45.10% [12], probably because the volunteers in studies had a similar average age at first smoking. Therefore, the group of volunteers has a concept or the same reason for starting to smoke.

4.2 Impact of the pandemic of COVID-19 in smokers

It was found that most result is a decrease income and more expenses. This is consistent with the previous studies Chakkrit et al, 2021 [13]. It was found that the most common impacts on people from the COVID-19 epidemic were income reductions and unemployment or out of business and the study of Supattra Runratt et al, 2020 [14] found that household incomes with all types of vulnerable groups (Except for the elderly) decreased during the coronavirus more than households without vulnerable groups while expenditure increased in proportion to the large decrease in income. This study collected the data after lockdown period, purchasing behavior and smoking behavior of the smoking population in Chiang Rai were stable. This is inconsistent with the previous studies, the study by Julia C. Chen-Sankey [15]. The smoking is more and has a tendency to quit smoking due to the epidemic situation of COVID-19 causing stress and anxiety [15]. They found that the population of tobacco use is increasing during the COVID-19 epidemic and during the lockdown period when they had increased stress and anxiety, feel alone and from long periods of quarantine, it has turned to using cigarettes to relieve stress [15-16].

4.3 Correlation between perception of severity of COVID-19 and self-protection behaviors from COVID-19

Poor correlation between perception of severity of COVID-19 and self-protection behaviors from COVID-19 of smokers was found in this study. The perception of severity of COVID-19 and self-protection behavior are not related. Though the data collection period, there is an announcement from the

Ministry of Public Health of the guidelines for preventing the spread of COVID-19 [17] through television and the internet and various channels. The smokers are not completely preventing COVID-19 following the guidelines.

There was an abnormal distribution indicating that the Chiang Rai smoking population had a high perception of the severity of COVID-19 (Median = 9/10 points). The distribution of self-defense behaviors data is normal. (Mean=15.1/20 points) shows that the smoking population in Chiang Rai province had variety of self-defense behaviors. It might be behaviors are based on knowledge and attitudes that drive behavior in which everyone [18].

Conclusion

COVID-19 impact to income and expense of smokers. The protection yourself by wearing a mask, wash your hands with soap or alcohol gel and keeping distance in Chiang Rai did not affect the smoking behavior and the purchase of cigarettes among the smoking population in Chiang Rai. The correlation between perceived severity of COVID-19 and self-protection behaviors during the COVID-19 epidemic in the smoking population Chiang Rai province found that there was poor. The population who smokes in Chiang Rai have variety of self-defense behaviors.

Limitation of this study

This study collected the data during Chiang Rai Province had number of COVID-19 patient less than previous, no country lockdown. Therefore, future studies it is recommended to study in other areas or study during other times when there is a different spread of COVID-19.

Recommendations for the future study

Covid- 19 pandemic in Thailand is getting better after a lot of people got COVID-19. Further study could study the effect of post COVID-19 in smokers who got COVID-19 whether impact on their perceived severity, economy, smoking behavior, or health.

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Effects of Extraction Solvents on Red Pigment, Phenolic Compounds and Antioxidant Capacity of Red Mold Rice Extracts

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ABSTRACT

Introduction: Red mold rice is the rice cultivated by *Monascus* species that has long been used as natural color in traditional foods. It contains various bioactive compounds and has high potential biological activities of such antioxidant, antimicrobial and anticancer activities. It could be interesting for extraction of bioactive compounds using various solvents.

Objective: This research was aimed to study effects of solvents on red pigment, phenolic compounds, and antioxidant capacity of red mold rice extracts.

Methods: Red mold rice was prepared by cultivating *Monascus purpureus* (*M. Purpureus*) under solid state fermentation method. The red mold rice was extracted with different 6 different solvents, i.e., DI water, 50% ethanol, 95% ethanol, 50% acetone, acetone, and ethyl acetate. Red pigment intensity was determined by colorimetric measurement. The total phenolic, and flavonoid contents were determined by Folin-Ciocalteu, and aluminum chloride colorimetric method, respectively. The antioxidant capacity of extracts was evaluated using DPPH and FRAP.

Results: The result revealed that solvent types had significant effect on red pigment and phenolic compounds extractions, as well as antioxidant capacity of red mold rice extracts ($p < 0.05$). The red mold rice extracted by ethyl acetate had the highest pigment intensity of $6,526.67 \pm 100.66$ unit/g extracts. Moreover, the highest total phenolic (78.13 ± 1.92 mg GAE/g extract) and flavonoid contents (97.10 ± 0.85 mg QE/g extract) was achieved by ethyl acetate while the lowest values were gained in the presence of water. The potential antioxidant activities expressed in terms of IC₅₀ and FRAP value were reached to 12.63 ± 1.01 μ g/mL, and 180.77 ± 7.70 mg TEAC/g extract, respectively, in the presence of ethyl acetate.

Conclusion: The properties of red mold extracts were strongly influenced by the type of solvent. Among of solvent tested, ethyl acetate was considered the potential solvent providing the highest red pigment, phenolic compounds, and antioxidant capacity. There are strong correlations among red pigment, phenolic compounds, and antioxidant capacity of red mold rice extracts.

Keywords: *Monascus purpureus*; solid state fermentation; ethyl acetate; pigments; biological activity

Introduction

Monascus spp. have long been used as natural substances through the long history in Asia. They belong to the group of Ascomycetes and particularly to the family Monascaceae. The genus *Monascus* includes four species: *M. pilosus*, *M. purpureus*, *M. ruber* and *M.*

froridanus, which account for the majority of strains isolated from traditional oriental foods. [1]. Among of them, *M. purpureus* is a popular strain because it possesses biological activities such as: antibacterial, antioxidant and antibiotic activities [2]. In addition, it can also produce red pigments that can be generally

produced on solid medium, especially steamed rice [3]. Red mold rice which is also known as Hongqu (in Chinese, “红曲”), red fermented rice, red yeast rice and angkak, is the fermented product of steamed rice inoculated with certain *Monascus* strains [4]. During fermentation, the red pigment is also produced by covering throughout the grains [5]. The red mold rice is composed of various chemical constituents, especially monacolins and pigments. In addition, organic acids, amino acids, sterols, decalin compound derivatives, lignans, coumarins, terpenoid, polysaccharides and flavonoid were reported as components contained in red mold rice [6]. It has been used extensively in Asia as a natural food colorant in Chinese cheese, red wine, sausage. Interestingly, it also has a potential as pharmaceutical substances and a great source of natural antioxidants [7]. In term of biological and pharmacological effects including antidiabetic effects, antifatigue effects, anti-inflammatory effects, antihypertensive effects, antihyperlipidemic effects, antioxidant effects, cholesterol-lowering effects [8] and anti-Alzheimer's effects [9]. Generally, the conventional techniques such as solid-liquid extraction have been widely used, mainly because of their efficiency, ease for manipulation [10]. Solid-liquid extraction is a method that separate one component from a mixture by dissolving it into solvent in which it is soluble while the other constituents of the mixture are not or less soluble [11]. It is generally known that the yield of extraction and biological activities of extracts depends on solvents and their concentration, the solvent/solid ratio, time of contact, temperature, and particle size of sample. Under the same conditions of extraction time, temperature and sample the solvent were found to be the important factors [12]. Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction; based on the solvent's polarity and the solubility properties of compounds contained in materials [13]. Many solvents have been served for polyphenols extraction [14]. Ethanol was good solvent for polyphenol, whereas methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols. mixture solvent of water and acetone was proper for extraction of higher molecular weight flavones [15]. For flavonoid compounds, aqueous ethanol solvent performed better than aqueous methanol and aqueous acetone [16]. Another solvent, ethyl acetate was found to help to extract both polar and non-polar compounds [17,18]. This research was aimed to investigate the effects of six solvents (water, 50% ethanol, 95% ethanol, 50% acetone, acetone and ethyl acetate) on red pigments and phenolic compounds of red mold rice prepared by solid state fermentation (SSF). Additionally, antioxidant activities of the red mold rice extracts were also determined.

Methodology

Microorganism

Monascus purpureus strain TISTR3615 was obtained from TISTR Culture Collection. The microorganism was sub-cultured on Potato Dextrose Agar (PDA) and incubated at 30°C for 10 days. The mycelium was then kept in refrigerator at 4°C until use.

Plant substrate

A 3/4 grain length of broken Jasmine rice was brought from local market. It was tightly collected in zip bags and kept in a closed plastic box at room temperature.

Solid substrate preparation

The experiment was carried out using 250 ml size Erlenmeyer flask sealed with cotton plug. A 25 g of broken jasmine rice was mixed with DI water at solid-liquid ratio of 3:2 w/v. The rice was soaked in water for 10 min. After that, the mixture was sterilized at 121°C for 15 min using autoclave, and then allowed to cool down.

Inoculum preparation

Spore suspension was prepared by cultivating *M. purpureus* on PDA plate for 10 days. A 2 ml of sterile Tween80[®] (0.1% v/v) was transferred to the culture plate. The *Monascus* colonies grown on the plate was scraped and suspended. After that, the suspension was filtrated using sterile cheese cloth. The concentration of spore was adjusted to 10⁶ spores/ml which was counted by using heamacytometer under microscope.

Solid state fermentation

Solid state fermentation (SSF) was performed according to Kritsadaruangchai et al. (2019) with some modifications [19]. Briefly, a 1.0 ml of spore suspension inoculum was added to the sterile solid medium, and then mixed well. The inoculated substrate was cultivated at 30°C for 10-14 days.

Preparation of red mold rice extracts

The fermented red rice was employed for solid-liquid extraction using 6 different solvents including water, 50% (v/v) ethanol, 95% (v/v) ethanol, 50% (v/v) acetone, acetone and ethyl acetate. The ratio of solid to liquid was 1:5 w/v. An extraction was carried out on orbital shaker with 125 rpm for 3 h. After that, it was filtered and then centrifuged at 8,500 rpm at 4°C for 20 min. The solvent was removed by rotary evaporator and/or freeze dryer. The obtained crude extracts were kept at 4°C until use for determining color pigment, UV wavelength scanning, phenolic and flavonoid contents, and antioxidant activities.

Determination of red pigment intensity

Red pigment intensity was determined by the method described by Konbangkerd et al., (2014) with some modification [20]. Briefly, the sample was dissolved with appropriate solvent at different concentrations. After that, the sample solution was determined at 505 nm using visible spectrophotometer. The pigment intensity was calculated using the following equation: Pigment intensity (unit/g dry weight) = ((A₅₀₅ x dilution factor x Volume of solution))/ (Weight of extract(g))

Determination of wavelength scanning

Red mold rice extract (1 mg) was solubilized into 10 mL of solution. The path length of the quartz cell used in this experiment was 1 cm. The absorbance spectrum was performed during 200 to 600 nm. [21].

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was determined by Folin-Ciocalteu method as described by Lee et al (2013) with some modifications [22]. Gallic acid was used as standard. Briefly, sample solution was mixed with 0.25 mL of Folin-Ciocalteu reagent and 1.5 mL of sodium carbonate solution (7.5% w/v) respectively. After 30 min, an absorbance at 765 nm was measured using visible spectrophotometer. The TPC was expressed as Gallic acid equivalents (GAE) per g of extracts.

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was analyzed according to the method by Lee et al (2013) with slight modification [22]. Sample solution was firstly mixed with 5% NaNO₂ (0.15ml), and then kept at ambient temperature for 5 min. The reaction was initiated by adding 4% NaOH (1.0 mL) and then stood for 8 min. An absorbance at 510 nm was measured using visible spectrophotometer. TFC was calculated according to a standard curve established with standard quercetin.

Determination of antioxidant activity by DPPH radical scavenging activity assay

The scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined according to the method described by Sanjukta et al (2015) with some modifications [23]. Trolox was used as standard.

Sample solution was mixed with 0.1 mM DPPH reagent, and then stood in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using visible spectrophotometer. The de-colorization of sample was calculated as the percentage of inhibition. The DPPH radical scavenging activity was expressed in term of milligrams Trolox equivalent antioxidant activity per gram extract (mg TEAC/g extract).

Determination of antioxidant by FRAP assay

The procedure was adapted from Benzie and Strain (1996) [24]. The reducing power of sample is based on the reduction of a ferric-tripyridyltriazine complex to produce a blue ferrous tripyridyltriazine. Trolox was used as standard. The sample was mixed with 0.3M acetate buffer (pH3.6) and FRAP reagent. The reagent was initiated by incubation at 37°C for 30 min and then the absorbance was measured at 593 nm using a visible spectrophotometer. The absorbance of a sample was also measured. The result was expressed in term of milligrams Trolox equivalent antioxidant activity per gram extract (mg TEAC/g extract).

Statistical analysis







All experiments were carried out at least in triplicate, and the result were presented as mean ± SD. One-way analysis of variance (ANOVA) was used to determine significant difference ($p < 0.05$), while Tukey's test for multiple comparisons, were also tested using SPSS software program (version 21).

Results

Pigment intensity

The red pigment intensity of red mold rice extracted by different solvents is represented in table 1. The results demonstrated that types of extraction solvent had significant effect on red pigment intensity of the red mold rice extracts ($p < 0.05$). The red mold rice extracted by ethyl acetate significantly displayed the highest red pigment value ($6,526.67 \pm 100.66$ unit/g extract), while the lowest red pigment intensity (53.33 ± 1.16 unit/g extract) when water was employed. In addition, it was clearly observed that the red pigment intensity of red mold rice extracts was inversely proportional to polarity of solvent.

Table 1 Red pigment intensity of red mold rice extracted by different solvents

Solvent	Illustration	pigment intensity (unit/g extract)
DI water		53.33 ± 1.16 ^a
50% Ethanol		650.67 ± 8.08 ^b
95% Ethanol		4,326.67 ± 83.27 ^c
50% Acetone		4,380 ± 69.28 ^c
Acetone		4,733.33 ± 50.33 ^d
Ethyl acetate		6,526.67 ± 100.66 ^e

Values are given as mean ± S.D. from triplicate.

Different letters in the same column indicate a significant difference ($p < 0.05$).

Wavelength absorption property

The wavelength scanning profiles of red mold rice extracts (0.1 mg/mL) are present in figure 1. The result revealed that ethyl acetate extract showed obviously different pattern of UV-visible absorption as compared with the other extracts. It strongly absorbed in the range of approximately 280-300 nm and 400-550 nm, while

water, 50% ethanol, and 95% ethanol extract showed a predominant absorption in the range of 200-300 nm. In contrast to acetone and 50% acetone extracts, absorption in the range of around 350-500 nm was only present. This indicated that solvents significantly affected absorption property in different UV and visible spectrum of red mold rice extracts.

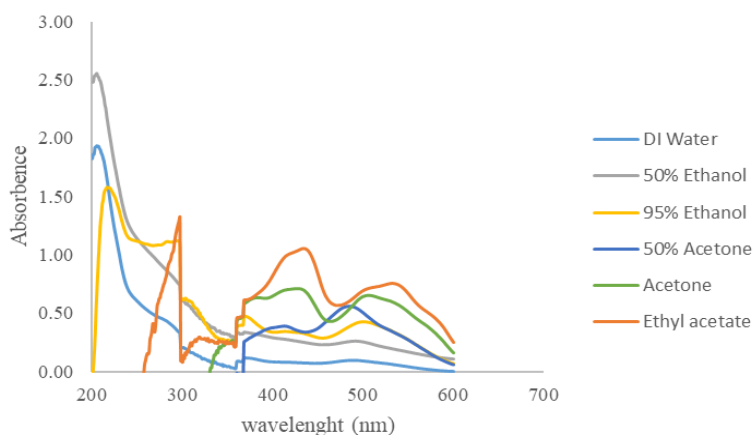


Figure 1 Wavelength scan in 200-600 nm of red mold rice extracted by different solvents

Total phenolic and flavonoid contents of the extracts from red mold rice using different extraction solvents are presented in table 2. The types of solvent had

Total phenolic and flavonoid contents

significant effects on phenolic and flavonoid extraction from red mold rice ($p < 0.05$). The ethyl acetate extract demonstrated the highest content of phenolic and flavonoid (78.13 ± 1.92 mg GAE/g extract and $97.10 \pm$

0.85 mg QE/g extract, respectively), while water was a poor solvent for extraction of phenolic (40.01 ± 0.09 mg GAE/g extract) and flavonoid (3.86 ± 0.06 mg QE/g extract).

Table 2 Total phenolic and flavonoid contents of red mold rice extracted by different extraction solvent.

Solvent	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
DI water	40.01 ± 0.09^a	3.86 ± 0.06^a
50% Ethanol	40.28 ± 0.84^a	26.50 ± 0.61^b
95% Ethanol	55.16 ± 1.40^b	59.55 ± 0.77^c
50% Acetone	47.79 ± 1.66^b	27.76 ± 0.63^b
Acetone	67.56 ± 0.77^c	73.47 ± 1.01^d
Ethyl acetate	78.13 ± 1.92^d	97.10 ± 0.85^e

Values are given as mean \pm S.D. from triplicate.

Different letters in the same column indicate a significant difference ($p < 0.05$).

Antioxidant activities

In this study, antioxidant capacity of the red mold rice extracts was evaluated based on 2 analytical methods including DPPH radical scavenging activity and FRAP. The free radical inhibition of the extracts was expressed as IC_{50} value ($\mu\text{g/mL}$), while the ability to reduce ferric ion was represented as Trolox equivalent antioxidant capacity (TEAC). The values of antioxidant activity of red mold rice extracts obtained for different solvents are represented in table 3. The results showed that the red mold rice extracted by ethyl acetate significantly showed the highest IC_{50} value of 12.63 ± 1.01 $\mu\text{g/mL}$ as compared with the other extracts. However, this IC_{50} value was approximately 4-fold higher than Trolox (3.66 ± 0.11 $\mu\text{g/mL}$). An increase of

IC_{50} value was apparently observed when higher polarity of solvent was present. The highest IC_{50} value was obtained in the presence of DI water (57.82 ± 2.93 $\mu\text{g/mL}$), indicating the lowest antioxidant capacity. This means that antioxidant activity of the red mold rice could be decreased when using solvent used for extraction was high polarity. The FRAP values had a similar trend to the DPPH results in which ethyl acetate provided the highest FRAP value (180.77 ± 7.70 mg TEAC/g extract), while the lowest value (81.21 ± 1.49 mg TEAC/g extract) was gained in 50% ethanol extract. Therefore, it can be confirmed that types of solvent strongly had significant effect on antioxidant capacity of red mold rice extracts.

Table 3 DPPH radical scavenging activity (IC_{50}) and FRAP of red mold rice extracted by different solvents

Solvent	DPPH radical scavenging activity (IC_{50} , $\mu\text{g/mL}$)	FRAP (mg TEAC/g extract)
DI water	57.82 ± 2.93^g	81.21 ± 1.49^b
50% Ethanol	32.34 ± 0.42^e	52.99 ± 0.17^a
95% Ethanol	41.08 ± 1.74^f	79.68 ± 2.31^b
50% Acetone	27.44 ± 1.27^d	105.22 ± 4.89^c
Acetone	17.73 ± 1.81^c	114.75 ± 3.81^c
Ethyl acetate	12.63 ± 1.01^b	180.77 ± 7.70^d
Trolox	3.66 ± 0.11^a	-

Values are given as mean \pm S.D. from triplicate.

Different letters in the same column indicate a significant difference ($p < 0.05$).

Discussion

The red mold rice extracts showed the increased red pigment intensity when extraction solvent used was low polarity. In this study, ethyl acetate displayed the highest red pigment value. The pigments of red mold rice synthesized by *M. purpureus* can be generally divided into orange (rubropunctatin and monascorubin), yellow (monascin and ankaflavin) and red (rubropunctamine and monascorubramine) [25]. Generally, red pigments are highly stable against pH, and high temperature

[26,27]. The *Monascus* pigments have a variety of physiological activities, such as antioxidant activity which is related to the pigment intensity [28]. So the pigment intensity could be an important indicator of the antioxidant activity of red mold rice extract. Ability to absorption UV-visible spectrum in the wavelength of 200-500 nm of red mold rice extracts was significant influenced by solvent used for extraction. This could be a results of different compounds represented in the different red mold rice extracts. In our studies, ethyl

acetate extract predominantly showed an absorption in the range of 350-550 nm. This could be relative with the presence of red pigment intensity in ethyl acetate extract. Koli et al. (2019) reported that combination of red pigment with chemical sunscreen increased the SPF by 36.53% and yellow pigment increased the SPF by 13.16%. The pigments may chemically interact with commercial sunscreen and undergo derivatization by making some changes in functional groups of chemical constituents present in sunscreen [29]. Phenolic components from natural sources have been suggested to play a role as natural antioxidants. Flavonoids are also potent antioxidants, free radical scavengers and they inhibit lipid peroxidation [30]. The different solvents used for extraction could lead to different compositions of phenolic compounds in extracts due to the different solubility of each phenolic compound. Consequently, the bioactivity of an extract might also be affected [31]. In this study, ethyl acetate was excellent solvent for extraction of total phenolic and flavonoids from red mold rice extract. Similar to the study of Hasim et al (2018), total phenolic and flavonoid contents in red yeast rice were highly in the presence of ethyl acetate [32]. Moreover, Zhu et al (2019) also reported that two main flavonoids found in red mold rice were daidzein and genistein [33]. The red mold rice extracts with high DPPH free radical scavenging activity exhibited higher protection against peroxidation [34]. In our study, red mold rice extracted by different solvents showed different antioxidant capacity. Among of them, the ethyl acetate extract displayed the strongest antioxidants based on the highest IC₅₀, and FRAP values. So, it can be implied that ethyl acetate was an excellent solvent for extracting an antioxidant from red mold rice extracts. Interestingly, the high correlations of red pigment intensity, phenolic and flavonoid contents, as well as antioxidant activities can be possibly emphasized that antioxidant capacity of the red mold rice extract could be resulted from phenolic compounds and red pigment represented in the extract. As reported previously, ethyl acetate showed to be the best solvent providing the lowest IC₅₀ value assayed by DPPH Kwon (2012) [35]. Therefore, type of solvents was considered the significant factor for assessing antioxidant capacity of the red mold rice produced by *M. purpureus*. The highest antioxidant activity of *Monascus* fermented rice can perform by various antioxidant actions including reducing power, DPPH radical scavenging ability, and ferrous ions chelating ability [36].

Conclusion

Type of solvent strongly affected red mold rice extract preparation. The highest red pigment, total phenolic and flavonoid contents, and antioxidant activities was gained when ethyl acetate was used as solvent as compared to acetone, 50% acetone, ethanol, 95% ethanol and water. An increase of solvent polarity tended to have the inverse effects. The ethyl acetate

extract highly provided red pigment intensity of $6,526.67 \pm 100.66$ unit/g extract, while phenolic and flavonoid contents were reached at 78.13 ± 1.92 mg GAE/g extract and 97.10 ± 0.85 mg QE/g extract, respectively. The highest values of IC₅₀ (12.63 ± 1.01 µg/mL) and FRAP (180.77 ± 7.70 mg TEAC/g extract) indicated the potential antioxidant capacity found in the ethyl acetate extract. Moreover, strong correlations among red pigment, phenolic compounds, and antioxidant capacity were remarkably present.

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The Effectiveness of 5% White Tea (*Camellia sinensis*) Cream for Periorbital Wrinkle Reduction

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ABSTRACT

Introduction: Although we cannot stop wrinkles formation, we can prevent and delay the process of formation. Wrinkle is the skin topographic changes due to aging which is the distinctive sign. Therefore, the researcher studied the effectiveness of white tea extract cream for periorbital wrinkle reduction. White tea extract contains a higher level of polyphenol which means higher anti-oxidant and anti-aging properties. The white tea extract is rich in polyphenols such as polycatechin, gallic catechin, epicatechin, epigallocatechin gallate (EGCG), epigallocatechin (EGC), gallic catechin gallate (GCG), epicatechin gallate (ECG), and flavanols, caffeine and gallic acid. It functions as an anti-oxidant by quenching the free radical and protects oxidative stress damage to the cells. It also inhibits elastase and collagenase activity and maintains elastin, collagen and delays skin aging process. So, it can treat fine lines, wrinkles and maintain elasticity of the skin. Besides, it prevents the occurrence of skin cancer, wrinkling and mottled pigmentation induced by photo damage.

Objective: To study the effectiveness of 5% white tea (*Camellia sinensis*) extract cream in periorbital wrinkle reduction

Methods: This research was a clinical controlled quasi-experimental study with 20 volunteers. The duration of the study was 12 weeks. Parameters such as wrinkle score by VISIA, Rao Goldman 5-point visual scale, cutometer score were used for assessment at baseline visit, 4th, 8th and 12th week.

Results: Mean cutometer scores at crow's feet and under eye area were significantly increased (p value < 0.001). Wrinkle evaluation score by VISIA was also statistically increased (p value < 0.001). No adverse effect was observed throughout the study.

Conclusion: The outcome of this study suggested that 5% white tea (*Camellia sinensis*) extract cream is effective for periorbital wrinkles reduction and safe for topical application. Hence, it could be an alternative anti-aging treatment for wrinkles reduction.

Keywords: *white tea extract: Camellia sinensis: periorbital wrinkles: elasticity: cutometer score*

Introduction

Skin is part of the human body which functions as a covering of the body. It covers 1.5-2 m² surface area of the body and weighs 15% of the total body weight., known as the largest organ of human body. Thus, it is the important indicator of aging.

Skin aging is the complex and multifactorial biological process. As the physiological process of the body changes with aging, skin also deteriorates with time. Skin aging can be mainly divided into two groups

– intrinsic aging and extrinsic aging.

Intrinsic aging is the inevitable general aging process which is also known as chronological aging. It can be influenced by genetic, hormonal changes and diversity of skin. It can cause pale skin with xerosis, fine wrinkles, laxity, production of collagen is reduced and more susceptible to infection and neoplasms. Functional changes are more distinct than morphological changes.[1]

Extrinsic skin aging is also known as external skin

aging and photoaging mainly due to environmental factors. This type of aging is preventable. The main cause of photoaging is ultraviolet (UV) light. Smoking, air pollution, exposure to chemicals, visible light, infrared radiation (IR) ozone, life style and diet also have an impact on extrinsic aging.

Among UV lights, major cause of photoaging is UVA and UVB. UVA can penetrate to the deeper layer of the dermis whereas 10% of UVB can penetrate up to the dermis. [2] Hormones also play an important role in skin aging. Decrease in estrogen level may lead to skin aging. It can reduce in collagen synthesis and elasticity, dryness. Skin becomes thinner, appearance of wrinkles and more fragile. [3]

People cannot stop the wrinkles formation because it is part of the normal aging process. Although we cannot stop, we can prevent its formation and delay the process of formation by avoiding the precipitating factors. Regular application of sunscreen and protection from UV radiation, maintaining healthy life style and consuming healthy diets and anti-oxidant rich food may help. Currently, there are varieties of anti-wrinkle treatments. Topical antioxidants such as tocopherols, ascorbic acid, polyphenols, flavonoids and green tea are well known. They can eliminate the free radicals and prevent harmful effects and photoaging. [4]

White tea is extracted from the leaves and leaf buds of *Camellia sinensis*. Tea have been widely consumed as a beverage by people all over the world for decades. *Camellia sinensis* plant is under the flowering plant family of genus Theaceae and it is originated from China and Asia. This plant is a hardy evergreen shrub or small tree with glossy green leaves and white to yellow flowers. It possesses fragrant, aromatic odor and prefers tropical and subtropical region. [5]

Tea can be subdivided into white tea, yellow tea, green tea, dark tea and oolong tea. They all are the products of *Camellia sinensis* plant with variations in processing method and oxidation level and harvesting time. White tea is the non-fermented tea with minimal oxidization process. White tea can be obtained only after sun withering and drying process. So, preparation methods have an important role for its properties. Recent studies show it has anti-inflammatory, antioxidant, antibacterial, anti-cancer properties and photoprotective properties. Apart from these, they are also used as weight controlling agent. [6]

Camellia sinensis contain many bioactive compounds. They are comprised with almost 4000 metabolites. [7] The major constituents are polyphenols of flavan-3ols

and flavanol which is more than one-third of metabolites and high contents of catechin and epicatechin (EC), Gallo catechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), gallic acid (GA), theaflavins and caffeine. [8] The bioactive compounds of teas are different depending on the fermentation and processing methods. Catechin contents and polyphenols are higher in white tea. It is said that level of processing has an inverse proportional to catechin contents. [6] Moreover, its caffeine and amino acid contents are higher than other teas.

Polyphenols in white tea scavenge reactive oxygen species (ROS), reactive nitrogen species (RNS) and thus reducing oxidative stress and showing great anti-oxidant activities. It scavenges singlet oxygen, superoxide-, hydroxyl-, peroxy-, and peroxy-nitrile radicals, chelates the trace elements and inhibits the enzymes which are involved in generation of ROS. [9] Hence, it prevents cellular inflammation induced by oxidative stress. Free radical imparts a crucial role in skin aging by triggering chain reactions and causing lipid, protein and nucleic acid damages.

Polyphenols can prevent oxidation of vitamin C and maintain the vitamin C level as well. This can maintain the skin elasticity because vitamin C functions as a coenzyme in collagen production. White tea extract can also halt the activity of metalloproteinase enzyme (MMP) and in turn prevents the destruction of collagen and elastin which are the important factors in aging. By inhibiting the elastase and collagenase, enhance the production of collagen to provide the skin elasticity and tensile strength. [6]

White tea is one of the herbal products and can be used for anti-aging purpose due to their high polyphenol content and health benefits. Apart from these, it can also protect from UV-induced damages, skin cancer and show photostability. Therefore, the researcher planned to study on anti-wrinkles action of white tea extract. There is no report of white tea on topical application for periorbital wrinkles reduction.

Objectives

General Objective

To study the effectiveness of 5% white tea (*Camellia sinensis*) extract cream in periorbital wrinkles reduction.

Specific Objectives

Primary objective

To compare the mean change of periorbital wrinkle reduction by 5% white tea (*Camellia sinensis*) extract cream from the baseline to different visit and week-12 visit, assessed by skin elasticity using Cutometer® MPA 580, VISIA® Complexion Analysis System and Rao-Goldman 5-point visual scoring scale.

Secondary objectives

1. To observe the adverse effects of 5% white tea (*Camellia sinensis*) extract cream.
2. To observe the participants' satisfactory score of 5% white tea (*Camellia sinensis*) extract cream.

Methodology

This research is a quasi-experimental controlled study with 20 participants and the duration is 12 weeks. The study population is healthy male and female between the age of 30-55 years old. They need to apply the 5% white tea extract cream on both sides of the periorbital area twice a day for 12 weeks. Follow-up appointments would be at 4th, 8th and 12th week. Volunteers were thoroughly explained about the research purpose, detailed procedure and anticipated risk and benefits of the study. Participants were requested to fill the form and sign the informed consent. Wrinkles assessment is done by Rao-Goldman 5-point visual score. Patch test for white tea (*Camellia sinensis*) extract cream was done prior to the study. Wrinkles and elasticity of the skin will be measured by VISIA® Complexion Analysis System and Cutometer® MPA 580. To measure the improvement, photographs taken with VISIA® Complexion Analysis System will be evaluated by 3 dermatologists. The participants were assessed by physicians and questionnaires to detect any adverse effects and participants' satisfaction. Ingredients are *Camellia sinensis* leaf extract, water, propylene glycol, butylene glycol, hydroxyethyl acrylate/ sodium acryloyldimethyl taurate copolymer, glycerin, hydrogenated polydecene, polysorbate 80, diazolidinyl urea, sodium Hyaluronate, iodopropyl butylcarbamate.

Sample Size Calculation

To calculate the sample size, use the reference data from a similar article, a study of the efficacy of *Ziziphus jujuba* extract for periorbital reduction by Shune,2021. Means and standard deviation were used to calculate the sample size.

Biophysical Techniques

The participants were photographed by using VISIA® Complexion Analysis System at baseline, 4th, 8th and 12th week.

The elasticity of the skin is also measured at baseline, 4th, 8th and 12th week. The elasticity is measured by using Cutometer® MPA 580.

Improvement and wrinkle score were evaluated by Rao-Goldman 5-point visual scoring scale and grading system.

Statistical Analysis

The data collected from the study was analyzed statistically by using SPSS software and Microsoft Excel 2019. This was carried out at Mae Fah Luang University Hospital, Bangkok Thailand.

Results

According to the demographic data, 15 of the participants were female and 5 of the participants were male with the mean age of 32.75±3.08 years. All of them have no history of other treatments before the study. Mean duration of exposure to sunlight between 10am to 4pm is 17±8.18 minutes. According to collected data, 10 volunteers have combination skin, 6 volunteers have oily skin and 4 volunteers have dry skin.

Results of Wrinkle Evaluation Score by VISIA

Table 1 Wrinkle evaluation score of 5% white tea extract eye cream by VISIA (n=20)

Grading Scale	5% white tea extract eye cream		
	Week 4	Week 8	Week 12
-1 = worse	0	0	0
0 = no changes	0	0	0
1 = 1-25% fair improvement	14	3	0
2 = >25-50% moderate improvement	6	17	15
3 = >50-75% good improvement	0	0	5
4 = > 75-100% excellent improvement	0	0	0

According to table 1, dermatologist scored fair improvement was seen in 14 volunteers and moderate improvement was seen in 6 volunteers. By 8th week, the majority (17 volunteers) were improved moderately and 3 volunteers were fairly improved. By 12th week,

moderate improvement was seen in 15 volunteers and good improvement was seen in 5 volunteers. Compared to the 4th week, more improvements were seen by 12th week.

Table 2 Statistical analysis of wrinkle evaluation improvement score by VISIA

	Mean	SD	p-value*
4 th week	1.30	0.47	
8 th week	1.85	0.37	<0.001*
12 th week	2.25	0.44	

Note: Data were analyzed within the group by the Friedman test. * p<0.05 is significant.

According to the statistical analysis from table 2, the mean wrinkle evaluation score with VISIA was increased and it was statistically significant (p<0.001). Evaluation score was improved and the mean scores were 1.30±0.47 in 4th week, 1.85±0.37 in 8th week and 2.25±0.44 in 12th week.

Table 3 Multiple comparison analysis of wrinkle evaluation score by VISIA at 4th, 8th and 12th week

Wrinkle evaluation by VISIA, means diff. (SD) / visit	Different Change	p-value*
8 th week-4 th week	0.6(0.1)	<0.001
12 th week-4 th week	0.9(0.1)	<0.001
12 th week-8 th week	0.4(0.1)	0.002

Note: Data were analyzed by a post hoc test. * p<0.05 is significant

According to statistical analysis of multiple comparisons of wrinkle evaluation scores, the mean change between 4th and 8th week is 0.6 and statistically significant (p<0.001) is seen. The mean change between 4th and 12th week is 0.9 and the difference is statistically significant too (p<0.001). The mean change between 8th and 12th week is 0.4 but it is not statistically significant.

Results of Dermatologists' Evaluation Score by Rao-Goldmann 5-point Visual Scale

Table 4 Dermatologists' evaluation score

Score	Week 0	Week 4	Week 8	Week 12
1=Wrinkle absent	0	0	0	3
2=Shallow but visible	2	8	16	16
3=Moderately deep	16	11	4	1
4= Deep wrinkle with well-defined edges	2	1	0	0
5=Very deep with redundant fold	0	0	0	0

At baseline visit, dermatologists evaluated 16 volunteers as score 3, 2 volunteers as score 2 and 2 volunteers as score 4. At 4th week, participants of score 2 were increased to 8 participants, score 3 was 11 participants and score 4 was 1 participant. At 8th week, dermatologists rated 16 participants as score 2 and 4 participants were score 4. In the 12th week, 3 participants achieved score 1, 16 participants were score 2 and 1 participant was score 3.

Table 5 Statistical analysis of dermatologists' evaluation score on baseline visit, 4th, 8th and 12th week

	Mean	SD	p-value*
Baseline	3.00	0.46	
4 th week	2.65	0.59	
8 th week	2.2	0.41	<0.001*
12 th week	1.9	0.45	

Note: Data were analyzed within the group by the Friedman test. * p<0.05 is significant.

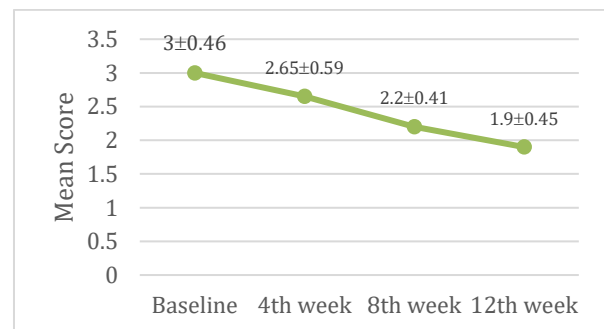


Figure 1 Linear graph revealing mean dermatologists' evaluation score at baseline, 4th, 8th and 12th week

According to table 5 and figure 1, mean dermatologists' evaluation scores were 3 ± 0.46 at baseline and 2.65 ± 0.59 at 4th week, 2.2 ± 0.41 at 8th week and 1.9 ± 0.45 at 12th week. The results decreased significantly ($p<0.001$)

Results of Cutometer Score for Elasticity of Skin

Table 6 Statistical analysis of cutometer score at crow's feet

	mean±SD	p-value*
Baseline	0.56±0.055	
4 th week	0.63±0.054	<0.001*
8 th week	0.71±0.050	
12 th week	0.80±0.059	

Note: Data were analyzed within the group by repeated measures ANOVA test. * p<0.05 is significant

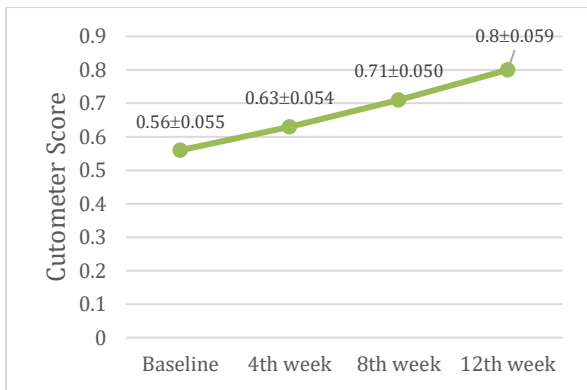


Figure 2 Linear graph revealing mean cutometer score at crow's feet on baseline, 4th week, 8th week and 12th week.

According to statistical analysis of cutometer score at crow's feet from table 6 and figure 2, mean cutometer score on baseline visit was 0.56 ± 0.055 . On the 4th week, the results were 0.63 ± 0.054 and on the 8th week it was increased to 0.71 ± 0.05 and on the 12th week the result was 0.80 ± 0.059 . The mean cutometer score was significantly increased at the level of 0.05. ($p<0.001$). Therefore, the mean cutometer score at crow's feet on 4th, 8th and 12th week were remarkably higher than the mean cutometer score at crow's feet on baseline visit.

Table 7 Multiple comparison analysis of cutometer score at crow's feet on 4th, 8th and 12th week

Cutometer score at crow's feet, mean diff. (SD) / visit	different change	p-value*
4 th week-Baseline	0.07(0.01)	<0.001
8 th week-Baseline	0.15(0.01)	<0.001
12 th week-Baseline	0.24(0.01)	<0.001
8 th week-4 th week	0.08(0.01)	<0.001
12 th week-4 th week	0.17(0.01)	<0.001
12 th week-8 th week	0.09(0.01)	<0.001

Note: Data were analyzed by post hoc test. * p<0.05 is significant

According to multiple comparison analysis of cutometer score by post hoc test from table 7, it was indicating statistical difference in each follow-up. Therefore, the difference in mean skin elasticity was significant when compared with different visits.

Table 8 Statistical analysis of cutometer score at under eye area

	mean±SD	p-value*
Baseline	0.54±0.080	
4 th week	0.64±0.079	<0.001*
8 th week	0.72±0.070	
12 th week	0.80±0.067	

Note: Data were analyzed within the group by repeated measures ANOVA test. * p<0.05 is significant.

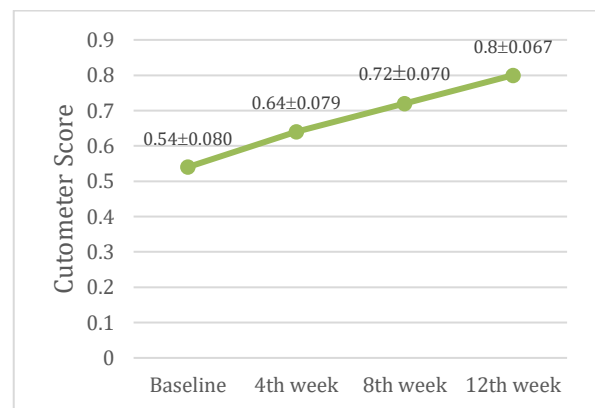


Figure 3 Linear graph showing mean cutometer score at under eye area on baseline, 4th week, 8th week and 12th week

Regarding to statistical analysis of cutometer score at under eye area from table 8 and figure 3, mean cutometer score were 0.54±0.08 on baseline visit, 0.64±0.079 on 4th week, 0.72±0.07 on 8th week and 0.80±0.067 on 12th week. The data indicated that the mean cutometer score was significantly increased at the level of 0.05. (p<0.001). Therefore, the mean cutometer score at undereye on 4th, 8th and 12th week were remarkably higher than the mean cutometer score at undereye on baseline visit.

Table 9 Multiple comparison analysis of cutometer score under the eye on 4th, 8th and 12th week

Cutometer score under eye area mean diff. (SD) / visit	Different Change	p-value*
4 th week-Baseline	0.09(0.01)	<0.001
8 th week-Baseline	0.18(0.01)	<0.001
12 th week-Baseline	0.26(0.02)	<0.001
8 th week-4 th week	0.09(0.011)	<0.001
12 th week-4 th week	0.161(0.01)	<0.001
12 th week-8 th week	0.08(0.01)	<0.001

Note: Data were analyzed by post hoc test. *p<0.05 is significant

According to multiple comparison analysis of cutometer score at under eye area from table 9, the mean difference was seen in different time points. The mean change in cutometer score at under eye area is statistically different in each week.

Participants' Satisfactory Score

Table 10 Participants' satisfactory score of 5% white tea (*Camellia sinensis*) extract eye cream on 12th week

Satisfactory Score	n (%)
Score 0 = No satisfaction	0
Score 1 = Little satisfaction	0
Score 2 = Moderate satisfaction	2 (10%)
Score 3 = More satisfaction	14 (70%)
Score 4 = Most satisfaction	4 (20%)

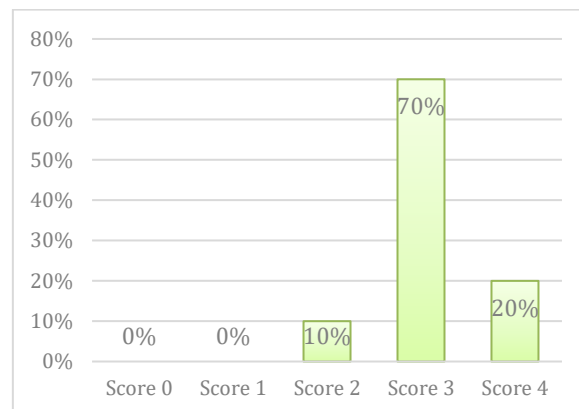


Figure 4 Bar chart exhibiting frequencies of participants' satisfactory score of 5% white tea (*Camellia sinensis*) extract eye cream on 12th week

Participants rated the satisfactory score on the 12th week. Regarding the collected data of table 10 and figure 4, the majority of the volunteers (n=14, 70%) rated more satisfaction, 2 volunteers (10%) rated moderate satisfaction and 4 volunteers (20%) rated most satisfaction.

Adverse Effects

In all participants, no adverse effect was detected throughout the treatment with 5% white tea (*Camellia sinensis*) extract eye cream.

Discussion

This was the study of 5% white tea (*Camellia sinensis*) extract eye cream for periorbital wrinkles reduction. In this study, 20 volunteers were recruited and all the volunteers came regular follow-up and completed the study. This was carried out in healthy male and female volunteers who were between 30-55 years old.

Improvement was measured with wrinkle evaluation score by VISIA. According to the statistical analysis, the results of mean wrinkle evaluation score with VISIA is increased statistically significant (p<0.001). When comparing the mean change from each visit, statistical different was seen between 4th and 8th week, 4th and 12th week (p < 0.001). However, the mean change between 8th week and 12th week was not statistically significant. The results were indicating that the improvement was increased in each follow-up. Rao-Goldman 5-point visual scale was also used to evaluate the wrinkles. Mean scores were reduced in each follow-up and it was statistically significant (p < 0.001). For the elasticity of the skin, cutometer was measured at two areas: crow's feet and under eye area. The mean cutometer score was significantly increased at the level of 0.05. (p < 0.001). Mean change of cutometer score at

crow's feet was compared for different visit and skin elasticity was improved significantly in each visit.

Regarding to statistical analysis of cutometer score at undereye area, the data were indicated that mean cutometer score was significantly increased at the level of 0.05. ($p < 0.001$). Therefore, mean cutometer score at undereye on 4th, 8th and 12th week were remarkably higher than the mean cutometer score at undereye on baseline visit. When comparing the mean change of undereye area's cutometer score from each visit, statistical different was seen.

In this study, increased in elasticity was seen significantly. This may be due to the action of white tea extract which halt the activity of metalloproteinase enzyme (MMP) and in turn prevents the destruction of collagen and elastin which are the important factors in aging [6]. Polyphenols in white tea can also prevent vitamin C oxidation and maintain skin elasticity. This is due to the fact that vitamin C functions as a coenzyme in collagen production. In this way, white tea extract can be used to treat fine lines and wrinkles and increase the elasticity of the skin. Therefore, this study is correlated with the previous study on maintaining skin elasticity and tensile strength [6].

Patients' satisfactory score was recorded on 12th week. Amazingly, 70% (14 volunteers) rated score 3 (more satisfaction) and only 10% (2 volunteers) rated score 2 (moderate satisfaction). 20% (4 volunteers) scored high satisfaction. Therefore, 5% white tea (*Camellia sinensis*) extract eye cream has high patients' satisfactory score. Throughout the study, no adverse effect was detected and the patch test did not show any allergic reaction. Hence, 5% white tea (*Camellia sinensis*) extract eye cream is safe for topical application.

Although intrinsic aging is inevitable, extrinsic aging can be prevented. Along with other wrinkles reduction lifestyles and treatments, topical use of eye cream can delay the process of wrinkles formation. This study revealed that 5% white tea (*Camellia sinensis*) extract eye cream is effective for periorbital wrinkles reduction. This may be due to its richness in bioactive compounds and antioxidants. High contents of polyphenol, epicatechin (EC), epigallocatechin gallate (EGCG) and other catechins might help in wrinkles reduction [6].

Polyphenols in white tea scavenge reactive oxygen species (ROS), reactive nitrogen species (RNS) and thus reducing oxidative stress and showing great anti-oxidant activities. It scavenges singlet oxygen, superoxide-

hydroxyl-, peroxy-, and peroxy nitrile radicals, chelates the trace elements and inhibits the enzymes which are involved in generation of ROS [9]. These anti-oxidant activities might enhance in delaying of aging process. This research indicated that it was correlated with the previous study about anti-oxidant action of white tea extract by scavenging free radicals and reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Previous study had proved that white tea can prevent damage to the Langerhans cells caused by UV radiation. UV radiation can provoke cutaneous immunosuppression which in turn leading to radiation of free radicals and enhance the aging process. [10,11] Polyphenols can lower UVB-induced inflammation and oxidative stress [12]. Hence, this may be the fact that white tea extract can increase elasticity and reduce wrinkles although no sunscreen was given in this study. Further study is required for photoprotective action to use as a photoprotective agent.

According to this study and results, 5% white tea (*Camellia sinensis*) extract eye cream is safe and effective for periorbital wrinkles reduction and also well tolerated. Any adverse effects such as erythema, skin irritation and hyper or hypopigmentation were not detected. For better results and efficacy, it should be used longer in further studies.

Conclusion

In regard to this study, the outcome of the mean change of cutometer scores at crow's feet and under eye area were significantly increased in different visits. Thus, 5% white tea (*Camellia sinensis*) extract could improve elasticity of the skin. Improvement was also statistically increased in follow-up visits. This study of 5% white tea (*Camellia sinensis*) extract also achieved high participants' satisfactory score and any adverse effect was not detected. To summarize, 5% white tea (*Camellia sinensis*) extract eye cream is effective for periorbital wrinkles reduction and safe for topical application. Hence, it can be an alternative anti-aging treatment for wrinkles reduction.

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Metadata and the Fatty Acids Content in Thai Shrimp Paste

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ABSTRACT

Introduction: The nutritional value of shrimp paste depends on the manufacturing process, such as the concentration of salt in the raw materials and the species of krill or small shrimp. In theory, different krill sources should have different bacterial species in communities. These are a major cause of the different chemical and physical properties of shrimp paste, especially the nutritional properties. This study focuses on microbial communities using the metagenome technique and the fatty acids content in the shrimp paste.

Objective: The metagenomic profiles of the bacteria and analysis of fat content in Thai shrimp paste.

Methods: Seven samples of fermented shrimp paste were obtained from various local markets and fishing villages and were analyzed for the fat content using an in-house method based on AOAC (2016). For analysis of the 16S rDNA metagenomic bacterial profile using Illumina sequencing.

Results: KK3 sample has the highest level of Saturated fatty acid and unsaturated fatty acid (Omega 3, Omega 6, Omega 9). A total of seven metagenomic samples were sequenced that report the dominant bacteria were *Bacillus*, *Lactobacillus*, *Staphylococcus*, and *Vibrio*.

Conclusion: This study can be the baseline information applied to develop the product of local or small enterprises.

Keywords: metagenomic profiles; shrimp paste; fatty acids

Introduction

Thai shrimp paste (kapi) is a fermented cuisine made primarily from salt, shrimp, or krill. Fermentation is a preservation method that uses a chemical transformation to turn raw materials into products via activities of an endogenous enzyme found in the raw material. The nutritional value of shrimp paste depends on the manufacturing process, for example, the concentration of salt and the species of krill or shrimp in the raw materials [1]. Proteins and lipids are hydrolyzed by natural microbial enzymes in krill. Microbes in krill survive in halophilic conditions, directly affecting total characteristics of shrimp paste [2].

This study is interested in three regions known for shrimp paste production in Thailand including the West coast of the upper Thai gulf, the East coast of the upper Thai gulf, and the lower Thai gulf. The raw materials for making shrimp paste on the West coast of the upper Thai gulf region using estuarine push net catching shrimp or krill from the deep-sea during December and used to preserve with salt in a ratio of about 10:1. For the East coast of the upper Thai gulf region using estuarine push net catching shrimp or krill in the estuary area and then put in the sun for drying raw material, which operates for preservation with salt in ratio about 8:2. Moreover, what is different is in the West coast of the upper Thai

gulf, shrimp or krill are caught using estuarine push net in the deep sea but on the East Coast of the upper Thai will use a estuarine push net to catch shrimp or krill around the estuary area. In addition, in the southern region, krill or shrimp are washed before being crushed. [3-5]

The shrimp paste contained a variety of fatty acids, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and Polyunsaturated fatty acids (PUFA) which are important sources for health benefits, especially PUFA fatty acid group (omega-3 and omega-6) that contribute significantly for human health associated with obesity, metabolic disease, insulin action, cardiovascular and immune functions [6]. Omega3 fatty offers beneficial effects on depression, rheumatoid arthritis, and asthma [7]. Additionally, alpha-linoleic acid (ALA) which is one of the omega-3 appears to benefit the heart, immune system, and nervous system [8]

According to Ali and colleagues (2020), shrimp paste contains essential amino acids more than non-essential amino acids and the fermentation period affects the concentration of amino acids and PUFA fatty acid group. The study found longer fermentation of shrimp paste resulted in a 6-week increase in essential oil production, almost equivalent to raw material (*Acetes* sp.), and an increase in PUFA production more than raw material [9]. Therefore, the food preservation process by fermenting shrimp paste has the potential to develop a healthy seasoning.

In theory, different krill sources should have bacterial communities containing varied species. These differences are a major cause of the different chemical and physical properties of shrimp paste, especially the nutritional properties. The growth of microorganisms depends on the raw materials used in the fermenting process [10]. The main raw material in shrimp paste was divided into two genera: *Acetes* and *Mesopodopsis* [11]. *Acetes* sp. is a genus of small shrimp with red tail, length 10-40 mm. Habitat in brackish and sometimes shallow coastal waters. Usually moved through the middle of the water or near the water surface, which was found in mangrove forests and seagrass, can be found from the surface of the water to a depth of about 20 meters. [12] *Mesopodopsis* sp. has general form long and slender and back eyes, length 10-30 mm. Habitat in coastal and brackish water areas. During the day, it will be in seabed and will float to the surface during the night [13]

The diverse types of shrimp paste will provide different nutrients because it may be the effect of the quantities of krill and the process of making shrimp paste differentially in each locality. However, there are enormous local shrimp pastes, for which the nutritional values and quality control processes have not been reported.

The metagenomic data from shrimp paste in Southeast Asia still lack data to support details of

spontaneous fermentation. Therefore, this study is interested in identifying the bacterial communities from local fisherman's villages in Thailand, using metagenomic next-generation sequencing, which is any of several high-throughput sequencing methods that can be used to directly identify the bacterial community from the sample without culturing [14].

Furthermore, to support the idea that "the different types of shrimp paste will provide different nutrients," the fatty acids content (omega-3, omega-6, and omega -9) of the samples where the types of shrimp used were identified in the previous study were measured.

Methodology

Fermented shrimp paste sampling

In three regions of the Thai gulf, Thailand (Figure1), seven samples of fermented shrimp paste were obtained from various local markets and fishing villages. The sampling areas were located and marked by GPS coordinates (Table 1) for further reference. Fermented shrimp paste samples were kept at 4°C in a refrigerator and metagenomic DNA was extracted within one month after sample collection. Totally seven fermented shrimp pastes were successfully collected and marked as KK1, KK2, KK3, SR1, SR2, SR3, and TP1.



Figure 1 The sampling areas in three regions of the Thai gulf, Thailand

Analysis of PUFAs and nutritional values in Thai fermented shrimp paste

Fermented shrimp paste samples were analyzed for the fat content including saturated fatty acids (SFAs): Polyunsaturated fatty acids (PUFAs) were measured using an in-house method based on AOAC (2016) 2003.05.

Metagenomic DNA extraction

DNA was extracted from shrimp paste using NucleoSpin® Soil kits (Macherey-Nagel, Düren, Germany). In the lysis process, lysis SL2 buffer and enhancer SX were used to increase DNA extraction efficiency. The concentration and purity of metagenomics DNA were measured by a 1.0% agarose gel electrophoresis and 260/280 nm absorbance using an Epoch microplate spectrophotometer (Bio-Tek). DNA samples with an A260/280 not less than 1.8 were collected at -20°C.

Table 1 Fermented shrimp paste sampling area

Shrimp paste samples	krill tissue (Previous study)	sampling area	GPS location
KK1	<i>Mesopodopsis orientalis</i>	Samut Songkhram	13°25'33.1"N, 99°58'41.7"E
KK2	<i>Acetes japonicus</i>	Samut Songkhram	13°25'28.1"N, 99°58'40.6"E
KK3	<i>A.japonicus</i>	Samut Songkhram	13°25'28.1"N, 99°58'40.6"E
SR1	<i>A.japonicus</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
SR2	<i>A.adonics</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
SR3	<i>A.japonicus</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
TP1	<i>A.japonicas</i>	Songkhla	6°52'12.4"N, 100°58'08.5"E

DNA analysis

Total metagenome DNA extracted from shrimp paste was sent to be analyzed for metagenome bacterial profile for the 16S rDNA V3-V4 region amplicon analysis [15] using Illumina sequencing (OMICS center, Chulalongkorn University)

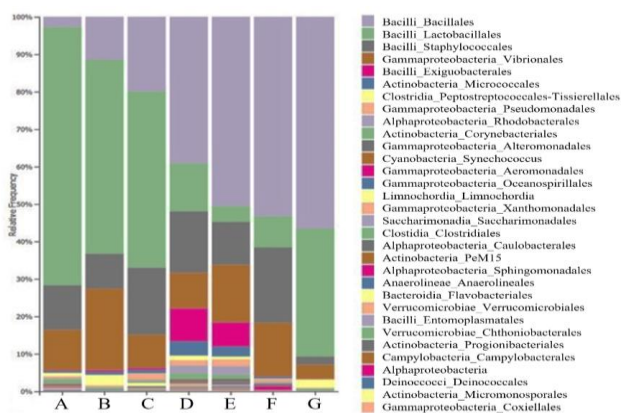


Figure 2 Metagenomic profile of Fermented shrimp paste from shrimp paste samples (A)KK1, (B)SR3, (C)KK3, (D)SR2, (E)SR1, (F)TP1, and (G)KK2.

Results

In addition, our previous research has identified krill species in Thai shrimp pastes (Table1), it was found that KK1 was reported as *M. orientalis* whereas KK2, KK3, SR1, SR2, SR3, and TP1 were reported as *A. japonicas*. The sample was collected from different areas of the Thai gulf but most of the shrimp found were identified as *A. japonicas*, which was inferred to be the habitat of this krill species in the Thai gulf.

Analysis of fatty acids in Thai fermented shrimp paste

The highest fat level was found in the KK3 sample from the province of Samut Songkhram, with a range of 1.35 to 3.17 g of fat per 100 g of sample. The percentage of saturated fatty acids ranged from 67.17 to 86.43 percent, with the highest concentration found in the KK1 sample and the percentage of unsaturated fatty acids ranged from 13.57 to 32.83 percent, with the highest concentration found in the KK2 sample from Samut Songkhram province (data not shown). On the other hand, when calculating the amount per 100 grams of shrimp paste, it was found that the KK3 sample has the highest level of Saturated fatty acid and unsaturated fatty acid (Omega 3, Omega 6, Omega 9) shown in Table 2.

Metagenomic analysis

A total of seven metagenomic samples were sequenced using next-generation sequencing (MiSeq; Illumina, San Diego, CA, USA). The dominant bacteria were *Bacillus*, *Lactobacillus*, *Staphylococcus*, and *Vibrio*. Furthermore, *Exiguobacterium* is also a dominant bacterium but was reported only in SR1 and SR2. The diversity of the bacterial community is shown in figure 2.

From the previous study, it was known that only KK1 from Samut Songkhram province used *M. orientalis* as the main raw material but the diversity of the bacterial community is shown that has the same pattern with KK2, KK3, SR1, SR2, SR3, and TP1.

Table 2 Fatty acids in Thai shrimp paste samples

Shrimp paste samples	Fat content (g/100g)	Saturated fatty acid (g/100g)	Unsaturated fatty acid (g/100g)	Omega 3			Omega 6		Omega 9
				ALA (g/100g)	EPA (g/100g)	DHA (g/100g)	LA (g/100g)	AA (g/100g)	OA (g/100g)
KK1	1.74	0.24	1.50	0.00	0.06	0.03	0.04	0.00	0.06
KK2	1.35	0.44	0.91	0.00	0.10	0.10	0.06	0.00	0.10
KK3	3.17	0.65	2.52	0.00	0.25	0.16	0.06	0.00	0.25
SR1	1.76	0.46	1.30	0.00	0.12	0.13	0.04	0.00	0.12
SR2	1.60	0.46	1.14	0.00	0.12	0.13	0.03	0.00	0.12
SR3	1.40	0.30	1.10	0.00	0.08	0.05	0.03	0.00	0.08
TP1	1.88	0.29	1.59	0.01	0.02	0.01	0.03	0.01	0.02

Discussion

Although the KK1, KK2, and KK3 were collected close to each other in the Samut Songkhram province, KK1 is used with different krill species from KK2 and KK3, but the diversity of the bacterial community showed the same pattern of microbial compositions. SR1, SR2, and SR3 samples are used *A. japonicus* that has the same raw material, only SR1 and SR2 have bacteria in phylum *Exiguobacterium*. These results suggest that the distinct species of krill will not directly provide to different microorganisms in shrimp paste, which may depend on the salt concentration used, length of fermentation, source of raw materials (shrimp habitat), and contamination in the process.

Omega 3 includes DHA and EPA fatty acids, an essential fatty acid that is recommended daily intake is 250-500 mg [16], The recommended daily intake of DHA in the human brain is 2.4–3.8 mg per day [17]. For Thai recommended daily intakes-Thai RDI suggests a daily intake of total fat is 65 g and recommended intakes for polyunsaturated fatty acids including the omega-3 and omega-6 range from 6-11 percent of your total daily energy intake (EPA and DHA combined 250-2000 mg per day), percent daily values are based on a 2000 calorie diet [18]. The shrimp paste is a useful source of DHA and essential amino acids. According to studies, a healthy ratio of omega-6 to omega-3 fatty acids appears to be between 1:1 to 4:1 [19].

Conclusion

This study is a novel investigation of the amount of omega-3, omega-6, and omega-9 in local Thai shrimp paste in comparative analysis. Furthermore, an analysis of the fatty acids in shrimp pastes also revealed that Thai shrimp paste is nutritious and healthy for healthy adults. Thai recommended daily intake of EPA and DHA is 250-2000 mg per day. From the analysis, it was found that most of the shrimp paste samples with 100 g of

essential fatty acids were sufficient to the recommended daily requirements. But in shrimp paste, there is also salt and calories that should be careful so this study provides fundamental data that can be used for the nutritional fact label and the precaution label. Moreover, reduction of salt in fermentation should be applied to produce healthier products. Therefore, this study reveals baseline information that can be applied to develop the product of local or small enterprises for further analysis to produce low-salt shrimp paste.

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Prevalence and Unit Cost of Abnormalities Detected in Abdominal Sonography among Soldiers

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ABSTRACT

Introduction: According to military doctrine, soldiers must maintain their health in order to remain strong and healthy at all times. Furthermore, they must be physically fitter than those in other professions.

Objective: To determine the prevalence of detected abnormalities and the unit cost of clinically significant abnormalities discovered during health screening by abdominal ultrasound (US) in soldiers. Study the prevalence of detected abnormalities and the unit cost of clinically significant abnormalities from health screening by abdominal ultrasound (US) among soldiers.

Methods: This was a retrospective study of 841 male and female soldiers who underwent abdominal US health screening at Chulabhorn hospital between February and September 2020. Data collection included abnormalities detected by ultrasound and diagnostic results classified by clinical significance into three categories: abnormality with no clinical significance, abnormality requiring follow-up, and abnormality requiring treatment. The unit cost for those abnormalities is calculated using the list of diagnostic procedures. The most common abnormalities in the United States are fatty liver, liver nodules, gallbladder polyps, renal stones, solid and cystic renal lesions, and prostate gland enlargement.

Results: There were 841 soldiers, 502 males and 339 females, with an average age of 51.8 years. There were 1,043 lesions detected in 643 (76.4 percent) soldiers. Fatty liver affects 364 (43.3 percent) of patients, liver nodules affect 46 (5.5 percent), gallbladder polyps affect 90 (10.7 percent), renal stones affect 41 (4.9 percent), solid and cystic renal lesions affect 128 (15.2%), and prostate gland enlargement affects 113 (22.5 percent). There were 79 abnormalities that were classified as requiring treatment. The unit cost was 19,007 Baht.

Conclusion: The prevalence of abnormalities detected by abdominal US in this study is comparable to that reported in the literature for the general population. As a result, the expectation that soldiers will have fewer abnormalities detected on US soil may not be realized.

Keywords: *ultrasound abdomen soldiers; prevalence of abnormalities; cost for ultrasound*

Introduction

The Sonographer School is an educational institution to provide health screening or diagnostic services by ultrasound. During the year 2020, a group of soldiers underwent whole abdominal ultrasound (US)

for health screening. According to the doctrine and strategies of the Royal Thai Army Education Department, soldiers need to have higher physical fitness than those of other professions. They have to maintain their health in order to always be strong and

healthy. Exercise intervention has been shown to reduce non-alcoholic fatty liver disease which could be detected by US screening [1]. Regular exercise and weight control is also effective in preventing gallstone formation [2]. However, no studies have been ever conducted in soldiers for the prevalence of detected abnormalities by abdominal ultrasound. Whereas, there are many other health conditions or diseases that may be detectable by abdominal US among soldiers. In fact, the military service personnel receive welfare from the government for their costs of health screening.

Methodology

This was a descriptive and retrospective study in both male and female soldiers who underwent abdominal ultrasound between February 2020 and September 2020 at the Sonographer School, Chulabhorn Hospital. Data collection included gender, age, body mass index, congenital disease, and US results of upper and lower abdomen, as well as diagnostic information of abnormalities detected by US. The diagnostic information of abnormalities was analyzed and classified according to clinical significance and criteria as follows: 1) Fatty liver – mild, moderate, and severe following the bright liver score [3], 2) Liver nodule – benign and malignancies, 3) Gallstone – gallstone without complications and gall stone with complications (US finding cholecystitis due to gallstone) [2,4], 4) Gallbladder polyps – following the size criteria, such as 0-5 mm, 6-9 mm, ≥10 mm and in case of the combined gallstone and gallbladder polyps [5,6], 5) Renal stone – following the size criteria, such as < 5 mm and ≥ 5 mm [7], 6) Solid renal tumor – benign and malignancies, 7) Cystic renal mass – Bosniak classification [8], 8) Enlarged prostate (≥30 ml) – post voiding residual urine volume < 50 ml and ≥ 50 ml [9]. In addition, other abnormalities with or without clinical significance were also categorized into 3 groups as follows: Group 1 Abnormalities without clinical significance, such as mild fatty liver, gallbladder polyps size 0-5 mm, renal

cyst Bosniak I, II. Group 2 Abnormalities which require follow-up, such as moderate fatty liver, benign liver nodule, gallstone without complications, gallbladder polyps size 6-9 mm, renal stone size < 5 mm, benign solid renal tumor, renal cyst Bosniak I, IIF, enlarged prostate (post voiding residual urine volume < 50 ml). Group 3 Abnormalities require treatments, such as severe fatty liver, malignancies liver tumor, gallstone with complications, gallbladder polyps size ≥10 mm, gallbladder polyp with detected gallstone, renal stone size ≥ 5 mm, malignancies solid renal tumor, renal cysts Bosniak III, VI, enlarged prostate (post voiding residual urine volume ≥ 50 ml). When grouping patients according to clinical significance and more than one abnormality is detected in each individual case, the most clinically significant abnormality group was considered. Descriptive statistics was applied to report general information of the study population, number of patients, and abnormalities detected. For unit cost calculation, only the direct medical costs incurred from the US and additional diagnostic costs in case that the results of such detected abnormalities should be confirmed and charged to patients according to reference prices from the Comptroller General's Department. The detected abnormalities in each group were calculated from the total costs incurred by diagnostic process in the following formula:

$$\text{The unit cost} = \frac{\text{Total costs}}{\text{Number of detected abnormalities}}$$

Results

According to the general information of 841 soldiers who received abdominal US between February 2020 and September 2020, it showed that the mean age was 51.83 ± 7.96 years and the mean BMI was 25.02 ± 3.57. High cholesterol was the most congenital disease in 206 soldiers, including others as shown in table 1.

Table 1 General information of patients

General information	Male	Female	Total
Number of patients	502 (59.7%)	339 (40.3%)	841 (100%)
Mean age (years) ± S.D.	54.25±5.82	48.25±9.24	51.83±7.96
Mean Body Mass Index ± S.D.	25.51±3.01	24.28±4.12	25.02±3.57
Number of high cholesterol	148 (29.5%)	58 (17.1%)	206 (24.5%)
Number of high blood pressure	86 (17.1%)	33 (9.7%)	119 (14.1%)
Number of diabetes	43 (8.5%)	17 (5.0%)	60 (7.1%)
Number of hepatitis B	2 (0.4%)	1 (0.3%)	3 (0.4%)
Number of thalassemia	0	1 (0.3%)	1 (0.1%)

The search results of the US report and diagnosis from abnormalities detected by the US yielded 643 patients (76.5%) including 404 males and 239 females. Whereas,

there were 198 patients (23.5%) with no detected abnormalities. The prevalence of detected abnormalities in soldiers was shown in table 2

Table 2 Prevalence of detected abnormalities in soldiers

Abnormalities	Number (percentage)		
	Male	Female	Total
Fatty liver	255 (50.8%)	109 (32.2%)	364 (43.3%)
Hepatic cysts	41 (8.2%)	34 (10.0%)	75 (8.9%)
Hemangioma or benign liver nodule	23 (4.6%)	22 (6.5%)	45 (5.4%)
Hepatocellular carcinoma	1 (0.2%)	0 (0.0%)	1 (0.1%)
Gallbladder polyps	65 (12.9%)	25 (7.4%)	90 (10.7%)
Gallstone	38 (7.6%)	34 (10.0%)	72 (8.6%)
Adenomyomatosis gallbladder	21 (4.2%)	17 (5.0%)	38 (4.5%)
Renal cysts	92 (18.3%)	26 (7.7%)	118 (14.0%)
Renal stone	26 (5.2%)	15 (4.4%)	41 (4.9%)
Angiomyolipoma	4 (0.8%)	6 (1.8%)	10 (1.2%)
Enlarged prostate gland	113 (22.5%)	0	113 (22.5%)*
Myoma uteri	0	74 (21.8%)	74 (21.8%)**
Abdominal aortic aneurysm	1 (0.2%)	0 (0.0%)	1 (0.1%)
Abdominal aortic atherosclerosis	1 (0.2%)	0 (0.0%)	1 (0.1%)

* Percentage per male patients only

** Percentage per female patients only

When studying details of each detected abnormality in patients with detected abnormalities and grouped according to clinical significance, the number of detected abnormalities with primary clinical significance and patients classified was in Group 3 or the group of abnormalities required for treatment for 79 lesions. Meanwhile, the number of 71 patients was classified into Group 3 required for treatment. Other detected abnormalities of secondary clinical significance and number of patients were shown in table 3.

According to the results of US, lesions requiring further diagnostic investigation included those found

nodules or a large mass in 16 subjects and lesions in the kidney including complicated cysts in 2 subjects and hyperechoic solid renal lesion in 1 subject. When classifying the list of diagnostics and price according to the Comptroller General's Department, the total costs were 1,501,600 baht as shown in table 4.

When calculating the unit cost of abnormalities categorized by clinical significance, it was found that the unit cost of abnormalities with no clinical significance or no required follow-up was 2,494 baht. The unit cost of abnormalities with required follow-up was 4,148 baht. The unit cost of abnormalities with required treatment was 19,007 baht as shown in table 5.

Table 3 Number of patients and number of detected abnormalities from abdominal US classified by clinical significance

Detected abnormalities classified by clinical significance	Number of patients (persons)/ Number of detected abnormalities (lesions)
Group 1 Detected abnormalities with no clinical significance or no required follow-up (Category I) Mild fatty liver, Hepatic cysts, Myoma uteri, Gallbladder Polyp size 0-5 mm, Adenomyomatosis gallbladder, Simple renal cysts, Abdominal aortic atherosclerosis	295 patients/ 602 lesions
Group 2 Detected abnormalities with required follow-up (Category II) Moderate fatty liver, Enlarged prostate gland (post voiding residual urine volume < 50 ml), Gallstones without complication, Hemangioma or benign liver nodule, Gallbladder Polyp size 6-9 mm., Renal stone size < 5 mm., Angiomyolipoma, Renal cysts (Bosniak IIF), Abdominal aortic aneurysm	277 patients/ 362 lesions
Group 3 Detected abnormalities with required treatment (Category III) Renal stone size ≥ 5 mm, Enlarged prostate (post voiding residual urine volume ≥ 50 ml), Severe Fatty liver, Gallbladder Polyp size ≥ 10 mm and Gallbladder polyp with gallstone detected, Gallstone with chronic cholecystitis, Hepatocellular carcinoma (HCC)	71 patients/ 79 lesions

Table 4 List and price of diagnostic radiology

List of diagnostic radiology	Price (baht)/ Time	Number of diagnostics (person/ time)	Total price (baht)
US upper abdomen	800	841	672,800
US lower abdomen	800	841	672,800
CT upper abdomen	6,000	5	30,000
CT whole abdomen	10,000	7	70,000
MRI upper abdomen	8,000	6	48,000
MRI liver	8,000	1	8,000
Total costs			1,501,600

Table 5 Unit cost of detected abnormalities classified by clinical significance

Abnormalities	Unit cost (baht)
Group 1 Detected abnormalities with no clinical significance or no required follow-up (Category I)	2,494
Group 2 Detected abnormalities with required follow-up (Category II)	4,148
Group 3 Detected abnormalities with required treatment (Category III)	19,007

Discussion

In this study, the prevalence of all abnormalities among soldiers showed that fatty liver was the most common abnormality, accounting for 43.3% and quite high when compared to other studies. For example, a study by Oshibuchi et al. examined the prevalence of abnormalities from upper and lower abdominal US in patients aged 20-60 years and fatty liver was noted in 15.52% [10]. Rungsinaporn and Phaisakamas studied the prevalence of abnormalities from upper abdominal US in patients aged 16-93 years and fatty liver was observed in 35.87% [11]. A study by Lee et al. on the prevalence of fatty liver disease among male soldiers, mean age 20.9 ± 1.3 years, showed fatty liver in 16.44% [12], with more attribution to the mean age of soldier group when compared to other studies in population with lower mean age or those with different age groups from teenagers to the elderly, which could be a factor for a higher prevalence of fatty liver disease. This was consistent with a study by Etmnani et al. which yielded a greatest prevalence of fatty liver in those aged 50-60 years when compared to other age groups [13]. For abnormalities in liver mass, the most common was hemangioma 5.4%, consistent with another study with a prevalence of 0.4-20% as the most frequent non-cancerous liver lesion cases [14] and 1 case of hepatocellular carcinoma (0.1%). The prevalence was not significantly different from a study with liver cancer found by abdominal US in the general population. In a study by Rungsinaporn and Phaisakamas, the prevalence of liver cancer was reported for 0.2%, while no report of liver cancer in a study by Oshibuchi et al [10,11]. The prevalence of gallbladder abnormalities included gallbladder polyps and gallstones for 10.7% and 8.6%, respectively in this study. The prevalence of polyps and gallstones in the general population was quite different in each country or area of study. However, the results of this study were consistent with another study in populations of East Asian countries, such as China, South Korea, Japan, etc., with the prevalence of gallbladder polyps for 4.3-12.3% and gallstones for 2.0-10.7% [15,16]. The prevalence of renal abnormalities, including kidney stones, was 4.9% in this study, consistent with a study in populations of Asian countries for 1.0-5.0% [17]. Meanwhile, the prevalence of mass lesion abnormalities, including angiomyolipoma, in this study was 1.2%, consistent

with another study of 0.3-3.0%, as the most common non-cancerous renal anomaly [18]. Other abnormalities included non-cancerous cysts in the kidneys, of which most results showed normal cysts. The prevalence of abnormalities in the lower abdomen among male soldier group was 22.5% for an enlarged prostate from the abdominal US in a study by Lepor et al. reported prevalence of enlarged prostate gland about 20% in males population among 40 to 60 [19]. The mean age of males was 54.25±5.82 years, this may result in higher prevalence of enlarged prostate, consistent with a study by Zhang et al. which stated a higher prevalence of enlarged prostate with increasing age [20]. The prevalence of abnormalities in the lower abdomen among female soldiers was only 21.8% for myoma uteri as benign abnormalities, consistent with a study by Dissaneevate which examined the effect of US in female subjects with no indications for US examination of the lower abdomen. [21]. Another significant abnormality in this study was abdominal aortic aneurysm (AAA), with a prevalence of 0.1% in a study by Dereziński et al. on the prevalence of AAA among rural and urban patients aged 60-65 years showed AAA in 0.82% and 1.01 in women group and men group, respectively [22]. The mean age of the population was 51.83±7.96 years, this may result in a small number of patients who detected AAA in this study. From the results of this study, it was found that the prevalence and type of abnormalities among soldiers detected by abdominal US are comparable to other populations in the report literature. Therefore, the expectation that soldiers should have less abnormality detected in the US than the normal population may not be true. For the incurred costs in this study, the results showed that the unit cost of abnormalities requiring treatment was 19,007 baht, which was calculated from the total costs incurred from the diagnostic procedures according to the number of clinically significant abnormalities requiring treatment. Different costs incurred from the US were caused by additional diagnostic radiography to differentiate suspected cancerous lesions of the liver and kidneys. The diagnosis showed 1 liver cancer. The costs per cancerous abnormalities in this study was 1,501,600 baht. However, despite other non-cancerous abnormalities, they could result in patient morbidity if left untreated, including Renal stone size ≥ 5 mm with possible urinary obstruction [7], Enlarged prostate gland

in males with post voiding residual urine volume. ≥ 50 ml, as an indication of urinary retention in the bladder [9], Gallstone with chronic cholecystitis, Gallbladder polyp size ≥ 10 mm, and GB polyp with gallstone detected as potential early cancer [5,6]. In this study, it could not state if it was worthwhile in health economics. The study provided an overview of the costs of health screening among soldiers compared to the detected abnormalities as a basis for considering the guidelines for the welfare and list of health check-ups in a group of military service personnel.

Conclusion

The health screening by abdominal US in this study among soldiers yields abnormalities that require both treatment and follow-up which are not much different from other populations reported in the literature.

Competing Interests

None

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Author contributions

The authors indicated in parentheses made substantial contributions to the following task of research Initial conception: conception and design (N.C.,S.S.); collection and interpretation of data (N.C.,S.P.S.); drafting the article or revising for important content (N.C.,S.S.); read and approved the final manuscript (N.C.,S.S.,C.C.,P.W.).

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Factors Affecting to Breast Cancer Screening for Breast Self-examination among Women in Phitsanuloke Province

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ABSTRACT

Introduction: Breast cancer is majority problems for women worldwide, especially in Thailand had incidence rate of breast cancer increasingly. Breast cancer screening can found early new patient.

Objective: Research aimed to study factors affecting to breast cancer screening for breast self-examination among women in Phitsanuloke Province, Thailand.

Methods: Cross-sectional study was carried out 16,202 women aged 35-60 years which be registered in Phitsanuloke Province, in fiscal year 2020, and sample size calculating used Daniel formula for 210 cases, systematic random sampling was determine. Data collected by questionnaires composed of 4 parts including, characteristics, knowledge, health perception, breast cancer screening. In addition to check questionnaire standardization, validity was checked by 3 experts and try out testing reliability about 0.86. Data analyzed using statistical as Pearson product moment correlation coefficient and Stepwise multiple regression analysis.

Results: Knowledge factors including knowledge of breast cancer, knowledge of breast cancer screening had mean score with high level ($\bar{x}=2.18$, S.D.=0.792), health perception including risk perceive, severe perceive, benefit perceive, problem perceive and confidence of breast self-examination had mean score with moderate level ($\bar{x}=2.46$, S.D.=0.49). Association analyzed found that knowledge of breast cancer, health perception was associated with breast cancer screening for breast self-examination among women with statistic significant ($r=0.208$, $P=.002$, $r=0.174$, $P=.012$, respectively). Prediction model analyzed reported five selected factors including; confidence of breast self-examination ($P<.001$), take information from village health volunteers ($P<.001$), take information from partner/family member ($P=.002$), no alcohol drinking ($P=.001$), benefit perceive ($P=.006$), respectively, overall selected factors accounted for 25.9% ($R^2=0.259$) of the variance breast cancer screening.

Conclusion: We should disseminate women' knowledge about initial symptoms of breast cancer, breast self-examination practice. Ministry of Public Health should provide the fundamental guideline for breast self-examination to health care providers at primary health care center to serve as a guideline for women.

Keywords: *Breast self-examination; Breast cancer screening*

Introduction

Breast cancer is a major health problem in worldwide, especially, the incidence rate of breast cancer occurred in both developed country and developing country [1]. The report from American cancer society showed the breast cancer accounting for 23% of all female cancer in 2002. It also accounted for

17.9% of all cancers for both sexes combined, which is the second highest cancer prevalence rate even when male cancers are included [2]. Breast cancer is not only an urgent public health problem in developed countries but it is also becoming an increasingly urgent problem in developing countries, where incidence rates have been increasing by up to 5% per year [3]. In Thailand,

breast cancer as the first leading cause of cancer among Thai women. In 2000 an estimated age-standardized incidence rate (ASR) for female breast cancer was 20.5 per 100,000 women. The highest incidence rate of female breast cancer was in Bangkok (ASR=24.3), and the lowest was in Nakhon Phanom (ASR=10.1) [4]. A particular concern is that the national incidence of newly diagnosed of breast cancer has been continuously increasing over the three year periods from 1992 to 2000. For the periods 1992-1994, 1995-1997 and 1998-2000 the national ASRs were 16.3, 17.2 and 20.5 per 100 000, respectively. [4,5,6] Furthermore, when Thai women are first diagnosed, they tend to have larger tumors and to be at a more serious stage of the disease. Cancer Registry data have shown that there is a high prevalence of late-stage disease (56% at Stage III and IV) when patients receive treatment [7]. Similarly, Angsusingh's study reported that 80% of Thai female breast cancer patients were detected at the advanced stage [8]. The late stage of disease and associated high mortality are seen as the result of delayed diagnosis and treatment [9]. Breast cancer screening can found early new patient. Ministry of Public Health have the breast cancer screening by 3 methods composed of breast self-examination: BSE, clinical breast examination: CBE, and mammography [10]. In present, we set the breast cancer screening by BSE for Thai women because BSE could have detected the breast cancer symptoms since in early stage about 80-90%. In additional, breast cancer patient who were diagnosed early stage (stage1 ,2), could be prompt treatment and increasing the survival rate 90%. Furthermore, the survival rate is increased in women diagnosed with the earliest stage of breast cancer (98%) as compared to women diagnosed with most advanced stage of 26% [11]. Hence, to reduce mortality from breast cancer, it is necessary to understand the factors causing the breast cancer screening for breast self-examination among Thai women in Phitsanuloke Province.

Objective

To study knowledge and health perception associated with breast cancer screening for breast self-examination among women in Phitsanuloke Province

Methodology

Cross-sectional study was carried out 16,202 women aged 35-60 year which be registered in Phitsanulok province, in fiscal year 2020. Sample size calculating used Daniel [12]. formula for 210 cases, systematic random sampling was determined.

Research instruments

Data collecting by questionnaires composed of 4 parts including,

Part 1: Characteristic of women were as follow: age, marital status, education completed, occupational, average family income, menstrual, child birth,

contraceptive used, health checking, family history of cancer, no smoking and no drinking.

Part 2: Knowledge variables were as follows: knowledge of breast cancer, knowledge for breast cancer screening. A categorical variable which had three categories, defined as "yes", "no" and "not sure". Scale items were composed of both positive and negative statements. For interpretation the score used Bloom theory [13] classified as 3 level such as; high level (score $\geq 80\%$) moderate level (score 61%-79%) low level (score $\leq 60\%$).

Part 3: Health perception variables were as follows: risk perceive, severity perceive, benefit perceive, barrier perceive and cues to action. Perceptions were measured on five-point Likert scales with response options ranging from strongly disagree, disagree, uncertain, agree, to strongly agree. Scale items were composed of both positive and negative statements.

Part 4: breast self-examination behavior variables were as follows: age to start BSE, frequency BSE, time for BSE practicing, step for BSE practicing, skill and confidence for BSE.

BSE were measured on five-point Likert scales with response options ranging from highest, high, moderate, low, lowest. Scale items were composed of both positive and negative statements.

For interpretation the score part 3 and part 4 follow as Best John W theory classified as 3 level such as; high level (score 3.67–5.00) moderate level (score 2.34–3.66) low level (score 1.00–2.33) [14].

The questionnaire was checked for completeness and satisfactory content validity and approved by 3 experts in breast cancer field. The reliability of the Likert scales was tested on 30 breast cancer patients and try out testing reliability about 0.86.

Data analyses

Data analyzed using statistical as Pearson product moment correlation coefficient and Stepwise multiple regression analysis.

Results

Table 1 shows the basic characteristics of the study population. Average age was 30-50 years (67.10%) (\bar{X} =46.18, S.D.=8.937, range 24-65 years). Most of the women (88.10%) were married or had a partner. Of the 166 women, 79% had primary education and 163 (77.60%) of women were agricultural workers. Average household income was 3,591.74 baht (US \$157) (\bar{X} =3,591.74, S.D.= 5,698.212, range 0-50,000 baht). For breast cancer screening by breast self-examination within 1 year, 128 (61%) women practiced for BSE at least one time per month and one-third (39%) do not practice BSE. Regard to breast cancer information sources, more than 67.60% of women received breast cancer information from public health volunteers, while

79% received breast cancer information from family member or partner

Table 1 General characteristics of women (n=210)

Characteristics	Number	Percentage
Age group (years)		
< 30	3	1.40
30 – 50	141	67.10
≥ 50	66	31.40
Mean = 46.18, S.D.=8.937, Min =24, Max =65		
Marital Status		
Single	4	1.90
Married	185	88.10
Widowed/Divorced	21	10.00
Education		
Not study	15	7.10
Primary school	166	79.00
Secondary school	23	11.00
Diploma	3	1.40
Graduate or higher	3	10.50
Occupation		
No work, house work	22	10.40
Company general employee	1	0.50
Government service	4	1.90
Business	2	1.00
Merchant	18	8.60
Agriculture worker	163	77.60
Average family income (Baht/Month)		
< 5,000	185	88.10
5,000 – 15,000	19	9.00
> 15,000	6	2.90
Mean =3,591.74, S.D.=5,698.212, Min = 0, Max = 50,000		
Alcohol drinking		
No	186	88.60
Yes	24	11.40
Receive breast cancer information		
No	38	18.10
Yes	172	81.90
Receive from partner/family member		
No	44	21.00
Yes	166	79.00
Receive from village health volunteers		
No	68	32.40
Yes	142	67.60
Practice for BSE		
No	128	61.00
Yes	82	39.00

Table 2 presents the percentages of patients with high, medium and low knowledge about breast cancer, and high, medium and low perceptions of its symptoms. Knowledge factors including knowledge of breast cancer, knowledge of breast cancer screening had mean

score with high level (\bar{X} =2.18, S.D.=0.792), health perception including risk perceive, severe perceive, benefit perceive, problem perceive and cues to action of breast self-examination had mean score with moderate level (\bar{X} =2.46, S.D.=0.499).

Table 2 Level of knowledge and health perception

Knowledge and health perception factors	Levels (%)			Mean	SD
	High	Moderate	Low		
1. Knowledge	41.90	34.30	23.80	2.18	0.792
- Knowledge of breast cancer	28.10	51.90	20.00	1.91	0.691
- Knowledge for breast cancer screening	61.00	19.00	20.00	2.41	0.803
2. Health perception	46.20	53.80	-	2.46	0.499
- Risk perceive	93.30	6.70	-	2.06	0.250
- Severity perceive	58.60	40.00	1.40	2.57	0.524
- Benefit perceive	83.30	16.70	-	2.83	0.373
- Barrier perceive	71.40	24.30	4.30	2.67	0.554
- Cues to action	38.60	60.50	1.00	2.37	0.504

Table 3 to investigate the associations of all factors and breast cancer screening by breast self-examination. The result showed that factor was association with breast cancer screening by breast self-examination with statistics significantly including; occupation ($r=0.147$, $p\text{-value}=0.034$), Receive from partner/family member ($r=0.244$, $p\text{-value}<0.001$), receive information from

village health volunteers ($r=0.260$, $p\text{-value}<0.001$) No alcohol drinking ($r=0.195$, $p\text{-value}=0.004$), knowledge for breast cancer screening ($r=0.263$, $p\text{-value}<0.001$), benefit perceive ($r=0.297$, $p\text{-value}<0.001$) barrier perceive ($r=0.195$, $p\text{-value}=0.005$) cues to action ($r=0.365$, $p\text{-value}<0.001$), respectively.

Table 3 Association between characteristics of women knowledge, perceptions and breast cancer screening by breast self-examination.

Variables	Breast self-examination	
	Correlation (r)	p-value
Characteristics of women		
- Age group	0.052	0.454
- Marital Status	0.019	0.789
- Education	0.107	0.122
- Occupation	0.147	0.034*
- Average family income	-0.043	0.533
- Receive from partner/family member	0.244	0.000*
- Receive information from village health volunteers	0.260	0.000*
- No alcohol drinking	0.195	0.004*
Knowledge	0.208	0.002*
- Knowledge of breast cancer	-0.052	0.457
- Knowledge for breast cancer screening	0.263	0.000*
Health perception	0.174	0.012*
- Risk perceive	-0.021	0.764
- Severity perceive	0.016	0.818
- Benefit perceive	0.297	0.000*
- Barrier perceive	0.195	0.005*
- Cues to action	0.365	0.000*

Note: * $p\text{-value}<.05$

Table 4 the prediction model analyzed reported five selected factors can predicted the breast cancer

screening by breast self-examination including; confidence of breast self-examination ($p\text{-value}<0.001$),

take information from village health volunteers (p -value<0.001), take information from partner/family member (P -value=0.002), no alcohol drinking (p -value=0.001), benefit perceive (p -value=0.006), respectively, overall selected factors accounted for 25.9% ($R^2 = 0.259$) of the variance breast cancer

screening. The prediction model follows as: $Y = 1.078 + [0.248 * \text{Cues to action}] + [0.199 * \text{Receive information from village health volunteers}] + [0.195 * \text{Receive information from health partner}] + [0.259 * \text{No alcohol drinking}] + [0.209 * \text{Benefit perceive}]$.

Table 4 Factors predicting breast cancer screening by breast self-examination

Predicting factors	B	S.E.	Beta	t	p-value
1. Cues to action	0.248	0.253	-	-4.265	0.000
2. Receive information from village health volunteers	0.199	0.063	0.256	3.957	0.000
3. Receive from partner/family member	0.195	0.065	0.191	3.066	0.002
4. No alcohol drinking	0.259	0.060	0.198	3.235	0.001
5. Benefit perceive	0.209	0.093	0.169	2.775	0.006
Constant	1.078	0.085	0.160	2.471	0.014
Constant = 1.078, F = 14.267, P-value<0.001, R = 0.509, R ² = 0.259					

Discussion

The prediction model analyzed reported five factors can have predicted the breast cancer screening by breast self-examination, overall selected factors accounted for 25.9% ($R^2 = 0.259$) of the breast cancer screening including; confidence of breast self-examination, take information from village health volunteer, take information from partner/family member, no alcohol drinking, benefit perceive, respectively. Confidence of breast self-examination is women felling to practice breast self-examination by themselves, and make sure to do this by step correctly. When the women practice breast self-examination, they have detected the abnormality on their breast area and know the criteria of abnormality symptoms. Similarly, the previous research found that women have the confidence of breast self-examination, it was associated the knowledge of breast cancer screening [15] and the research from Holmberg [16] reported that 98.5% of women who do not practice breast self-examination every monthly because they don't know the step of breast self-examination, so they don't have the confidence of breast self-examination. Information about the breast cancer and breast cancer screening was associated with breast self-examination because the mass media is the main factor to support all women received the information of breast cancer, information of breast cancer screening processing and the step technique for breast self-examination. Thus, women received the information about breast cancer from variety sources, for example, television, radio, newspaper, poster, family member/partner, health workers, village health volunteers, conference meeting. In additional, the information have many sources for women to search the breast cancer data but the information about breast cancer screening and breast self-examination not enough [17]. Because women who do not receive the breast cancer screening information,

maybe they don't know the technique for breast self-examination practicing. The research result showed that women received information from village health volunteer (67.60) and received information from partner/family member (79%), respectively. In this reason, the village health volunteers, family member or partner are very necessary to provide and support women to search the breast cancer information, especially how to do the breast self-examination. Because the village health volunteer is representing from health worker who worked in community and nearly all people in community. Moreover, village health volunteers are the key person to provide the knowledge and skill for breast self-examination practicing. The women' characteristic factor was associated with breast self-examination every monthly. Many research showed the evidence based to support that the characteristic of women effecting to breast self-examination with statistic significantly including no alcohol drinking, similarly the research by AL-Naggar [18]. Health perception about breast cancer symptoms is important factors, which influence help-seeking behavior and breast self-examination practicing, especially, the benefit perception. Other the benefit perception reasons related to breast self-examination practicing have been that women are scared of the diagnosis and surgery, and having to wait for an appointment [19]. Health perception towards seeking help can also be important factors affecting breast self-examination practicing. It has been found that women, who breast self-examination, are more reluctant to bother their GP, and they are more likely to express explicit fears about the consequences of diagnosis and treatment of the disease [20].

Conclusion

As a result, reported 5 factors affecting to breast cancer screening for breast self-examination among

women including; confidence of breast self-examination, take information from village health volunteers, take information from partner/family member, no alcohol drinking, benefit perceive, respectively. In order to, the researcher will be applied all factor to create the breast-self-examination motivation program for women practicing for every monthly.

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Effect of Thai herbal recipes on the immune system in streptozotocin-induced diabetic retired breeder mice

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ABSTRACT

Introduction: The deterioration of the immune system occurs with increasing age. Additionally, impaired insulin secretion and decreased insulin sensitivity are associated with aging. Type 2 diabetes mellitus is related to immune dysfunction that causes the elderly with diabetes to have a higher risk of infection. Herbal-based treatments are one of the therapeutic strategies for reducing blood glucose and also rejuvenating the immune system to improve the quality of life of the elderly.

Objective: To investigate the immunomodulatory effect of two Thai herbal recipes in streptozotocin (STZ)-induced diabetic aging mouse model. Recipe 1 consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* (1:1:1). Recipe 2 substituted *C. zeylanicum* for *G. inodorum*.

Methods: Thirty-week-old male mice with diabetes induced by STZ were orally administered with Thai herbal recipes at the doses of 250, 500, and 1,000 mg/kg for 28 days. Blood glucose monitoring was performed on days 7, 14, 21, and 28. After 28 days, mice were sacrificed, spleen and thymus were weighed. The peripheral white blood cell count and differential were measured.

Results: Treatment with both herbal recipes lowered blood sugar levels, however, the body weight loss could not improve in diabetic aging mice. Relative thymus weight seemed to increase in diabetic aging mice treated with a low dose of herbal recipe 1 and a high dose of herbal recipe 2. Additionally, diabetic aging mice treated with a low dose of herbal recipe 1 significantly increased relative spleen weight, whereas treatment with herbal recipe 2 at low and middle doses tended to decrease relative spleen weight. Treatment with both herbal recipes caused the diabetic aging mice to have a lower peripheral monocyte count. Nonetheless, treatment with a high dose of herbal recipe 1 significantly increased peripheral white blood cell (WBC), lymphocyte, and basophil counts.

Conclusion: This study demonstrated the immunomodulatory and hypoglycemic effects of two Thai herbal recipes in diabetic aging mice. Interestingly, Thai herbal recipe 1 tended to be able to boost the immune system.

Keywords: Thai herbal recipes; Immunomodulatory; Aging; Immune system; Mice

Introduction

According to the World Health Organization (WHO), the global population aged over 60 years will

increase from 12% to 22% between 2015 and 2050. Importantly, more than 80% of the elderly are predicted to live in low- and middle-income countries by 2050 [1].

Aging is associated with an increase in molecular and cellular damage, resulting in multiple system dysfunctions of the body including the immune system. Further, Type 2 diabetes mellitus (DM) is becoming one of the most life-threatening problems in the elderly. According to the latest estimates of the International Diabetes Federation (IDF), the diabetic people aged 65-99 years were estimated to be 123 million (around 18% of prevalence rate) in 2017 and are expected to double in 2045 [2]. Furthermore, the global population with DM aged 20-79 years were estimated to be 536.6 million (around 10.5% of prevalence rate) in 2021 [3]. Type 2 DM is linked to immune dysfunction that causes the elderly with diabetes to have a higher risk of infection. The immune system is a critical host defense system against invading pathogens. Aging-related immune changes have been investigated in several studies, and the results indicated that there were both quantitative and qualitative alterations of the immune system during senescence [4-6]. A decrease in immune functions causes the elderly to become more vulnerable to infectious diseases and other age-related diseases such as cancer and neurodegenerative diseases [5]. Therefore, preventing or boosting the immune system is the proper way that can improve the quality of life for the elderly. The previous studies have reported many species of Thai herbs such as *Momordica cochinchinensis*, *Clitoria ternatea*, and *Cinnamomum zeylanicum* that demonstrated immune-enhancing activity in both *in vitro* and *in vivo* studies [7-9]. Additionally, there are studies that have shown the antidiabetic activity of *M. cochinchinensis*, *C. ternatea*, *Gymnema inodorum*, and *C. zeylanicum* [10-13]. Nevertheless, there are no scientific reports about the effect of the recipe using these herbs on the immune system in diabetic animals. Thereby, we had invented an herbal recipe with 3 types of plants consisting of *M. cochinchinensis*, *C. ternatea* and *C. zeylanicum* that have immune-enhancing and antidiabetic activities. Since *G. inodorum* is a plant found mostly in northern Thailand and has antidiabetic properties, thus, we were interested in using it as a substitute for *C. zeylanicum* in the herbal recipe. The aims of this are to investigate the immunomodulatory effect of two Thai herbal recipes (recipe 1 consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* at the ratio of 1:1:1, and recipe 2 substituted *C. zeylanicum* for *G. inodorum*.) in diabetic models of male retired breeder mice.

Methodology

Preparation of the Thai herbal recipes

All plants were collected from Chiang Mai, Thailand, and identified by Asst.Prof.Dr. Sunee Chansakaow at Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University. Briefly, dried aril of *M. cochinchinensis* was ground with corn flour and Cab-O-Sil to obtain a homogeneous mixture. Flowers of *C. ternatea* in distilled water were boiled for

5 min. The extract was filtered, added with Cab-O-Sil, and then evaporated to obtain the dry extract. The aerial parts of *G. inodorum* in distilled water were boiled for 1 h. Next, the extract was filtered and repeated boiling two times. Then, Cab-O-Sil was added, and the mixture was evaporated to dry the extract. The dried stem bark of *C. zeylanicum* was ground to be a fine powder. Subsequently, two herbal recipes were prepared by mixing the three plant extracts in a ratio of 1:1:1. The first recipe consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum*, while the second included *C. zeylanicum* in place of *G. inodorum*.

Experimental animals and housing

ICR mice (30-week-old males weighing 40-60 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom, Thailand. The mice were housed in individually ventilated cages, fed with a standard pellet diet and water *ad libitum*. Experimental animals were conducted according to the regulations of the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (21/2563).

Experimental design

Blood samples were collected from the tail vein by snipping the tail tip to measure the blood glucose levels at baseline by a glucometer. The mice fasted overnight for 20 h before STZ administration, a single *i.p.* injection of 80 mg/kg in 0.05 M citric acid buffer, pH 4.5, to induce diabetes [14]. Seven days following STZ administration, blood collections from a tail vein were performed before starting the administration of the test substance. The male ICR mice were randomly divided into eight groups ($n = 5$ per group). Group 1 (control group) was treated with distilled water; Group 2 (reference group) was treated with metformin (400 mg/kg body weight); Groups 3-5 were orally gavaged with herbal recipe 1 (250, 500, and 1,000 mg/kg body weight, respectively); Groups 6-8 were treated with herbal recipe 2 (250, 500, and 1,000 mg/kg body weight, respectively). All mice were orally gavaged once daily for 28 days. Blood glucose levels were measured at 60, 120, and 240 min after the first treatment. After that, blood glucose monitoring was performed on days 7, 14, 21, and 28. Body weight was measured before and after STZ injection and on days 7, 14, 21, and 28. After sacrifice, blood samples were collected for WBC counts and differential, and the spleens and the thymuses were collected and weighed. Relative organ weights were calculated as organ weight (mg)/body weight (g).

Results

Effect of Thai herbal recipes on blood glucose levels in diabetic mice during 28 days of administration

Average blood glucose levels of all groups of mice were in the range of 80 to 110 mg/dL before the STZ injection. Seven days after the STZ injection, the

average blood glucose levels of mice increased to a range of 430 to 450 mg/dL. These results demonstrated that a single injection of 80 mg/kg STZ caused the mice to have higher blood glucose levels and develop diabetes. There was no statistically significant difference in blood glucose levels between groups before and after the STZ injection. Seven days after repeated-dose treatment, the diabetic control mice showed an increasing trend in blood glucose levels, while the blood sugar level of the metformin-treated group tended to decrease but was not statistically

different when compared to the diabetic control group. Moreover, the reduction in blood sugar level after 7 days of administration was also observed in all groups of diabetic mice treated with herbal recipe 1 and the diabetic mice treated with herbal recipe 2, however, there was no significant difference when compared to the diabetic control group. The effect of both herbal recipes in lowering the blood sugar level was not dose-dependent. Nevertheless, it was worthwhile noting a progressive severity of diabetes observed after day 7 and caused some death in mice (table 1).

Table 1 Blood sugar levels after STZ injections and during administration of Thai herbal recipes. Data are represented as mean ± SEM (n=5 mice per group at the beginning and n=2-4 mice per group after day 7).

Group	After STZ injection (mg/dL)	Treatment			
		7 day (mg/dL)	14 day (mg/dL)	21 day (mg/dL)	28 day (mg/dL)
Control	447.20±63.85	468.00±72.58	481.40±79.30	503.33±80.27	578.50±21.50
Met (400)	441.40±58.34	381.50±65.99	481.33±90.11	486.67±47.35	515.00±85.00
R1 (250)	444.40±50.70	383.75±76.60	408.67±34.37	373.50±4.49	425.00±4.00
R1 (500)	432.40±54.91	407.80±62.38	336.60±61.31	425.00±58.53	467.50±46.72
R1 (1,000)	448.40±31.53	425.00±55.35	448.33±45.06	478.00±47.06	551.33±20.25
R2 (250)	426.20±50.10	349.40±55.02	400.25±112.93	522.00±31.77	548.00±23.00
R2 (500)	432.60±54.62	434.50±35.50	387.00±58.65	543.33±25.62	442.00±79.10
R2 (1,000)	430.20±58.48	381.00±60.58	373.75±62.75	356.33±79.44	478.50±4.50

Table 2 Blood sugar levels at different time points after single-dose administration of Thai herbal recipes. Data are represented as mean ± SEM (n=5 mice per group).

Group	Blood glucose level (mg/dL)			
	0 min	60 min	120 min	240 min
Control	447.20±63.85	488.00±51.72	496.60± 5.14	485.00±58.86
Met (400)	441.40±58.34	424.00±55.66	384.60±66.75	327.60±87.24
R1 (250)	444.40±50.70	441.80±52.22	438.00±27.32	354.60±57.65
R1 (500)	432.40±54.91	435.00±47.07	437.80±52.12	373.60±68.40
R1 (1,000)	448.40±31.53	462.80±23.20	457.20±23.12	407.00±25.38
R2 (250)	426.20±50.10	476.40±24.63	422.00±36.65	376.60±38.98
R2 (500)	432.60±54.62	455.60±29.42	416.40±27.53	411.40±11.16
R2 (1,000)	430.20±58.48	424.60±87.18	395.80±93.15	371.00±82.82

Effect of Thai herbal recipes on blood glucose levels in diabetic mice after single-dose administration

As demonstrated in table 2, the diabetic control group had increased blood sugar levels at 60 and 120 minutes after distilled water administration and slightly decreased at 240 minutes, whereas the metformin-treated group showed a decrease in blood sugar level after 60 to 240 minutes of administration. The blood sugar levels of the diabetic mice treated with herbal recipe 1 and recipe 2 tended to reduce obviously at 240 minutes after administration. Nonetheless, there was no statistically significant difference among groups at any time point.

Effect of Thai herbal recipes on body weight in diabetic mice

The average body weights of mice before the STZ injection were in the range of 49 to 52 g and decreased to 41 to 45 g within 7 days after the STZ injection (table 3). These results indicated that a single injection of 80 mg/kg STZ resulted in the loss of body weight in mice. In the diabetic control mice, the body weight was inclined to reduce throughout the study. The body weight of the metformin-treated mice was steady during metformin administration. The same manner was observed in the groups that received herbal recipe 1 (1,000 mg/kg) and recipe 2 (500 mg/kg). However, there was no statistically significant difference in body weights among groups at any time point.

Table 3 Body weights after STZ injections and during administration of Thai herbal recipes. Data are indicated as mean \pm SEM (n=5 mice per group at the beginning and n=2-4 mice per group after day 7).

Group	Body weight (g)				
	After STZ injection	7 th day	14 th day	21 st day	28 th day
Control	41.60 \pm 1.72	40.00 \pm 1.55	38.80 \pm 1.02	37.00 \pm 0.58	37.33 \pm 2.91
Met (400)	42.40 \pm 2.23	41.00 \pm 1.00	42.67 \pm 2.67	42.67 \pm 1.76	42.00 \pm 2.00
R1 (250)	42.40 \pm 1.17	39.00 \pm 1.00	39.00 \pm 1.29	37.33 \pm 1.76	37.33 \pm 1.76
R1 (500)	44.40 \pm 2.79	39.60 \pm 1.47	41.60 \pm 1.72	40.00 \pm 3.74	41.50 \pm 2.06
R1 (1,000)	43.20 \pm 1.50	40.00 \pm 3.90	40.50 \pm 2.63	41.33 \pm 2.40	42.00 \pm 4.16
R2 (250)	44.40 \pm 2.64	37.20 \pm 2.58	38.00 \pm 1.41	38.50 \pm 1.50	38.00 \pm 2.00
R2 (500)	42.80 \pm 1.36	40.50 \pm 4.11	42.00 \pm 2.00	42.67 \pm 2.91	42.00 \pm 3.06
R2 (1,000)	41.00 \pm 1.91	36.00 \pm 1.63	35.00 \pm 1.73	33.00 \pm 2.65	34.00 \pm 2.31

Effect of Thai herbal recipes on relative thymus weight in diabetic mice

After 28 days of treatment, the relative thymus weights of the diabetic mice treated with herbal recipe 1 at the dose of 250 mg/kg and herbal recipe 2 at the dose of 1,000 mg/kg tended to be increased, whereas the treatment with herbal recipe 1 at the doses of 500 and

1,000 mg/kg was similar to that of the control group. A slight decrease in relative thymus weights was found in diabetic mice treated with metformin and in diabetic mice treated with herbal recipe 2 at the doses of 250 and 500 mg/kg as shown in figure 1. Nevertheless, there was no significant difference in relative thymus weights among groups.

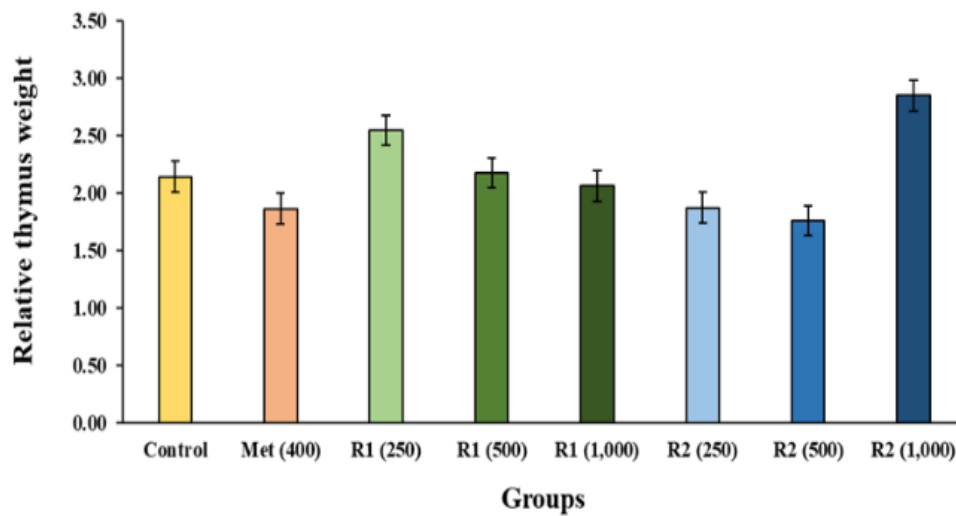


Figure 1 Relative thymus weight of diabetic mice after the administration of Thai herbal recipes for 28 days. Bar graph showed mean ± SEM (n=2-4 mice per group).

Effect of Thai herbal recipes on relative spleen weight in diabetic mice

A significant increase in the relative spleen weight was found in the group of diabetic mice treated with herbal recipe 1 at the dose of 250 mg/kg when compared with the diabetic control mice. Other groups consisted of diabetic mice treated with metformin, herbal recipe 1 at the doses of 500 and 1,000 mg/kg, and herbal

recipe 2 at the dose of 1,000 mg/kg tended to be increased in relative spleen weights but not statistically different when compared to the diabetic control group. The relative spleen weight in groups that received herbal recipe 2 at the doses of 250 and 500 mg/kg was decreased without statistically significant difference when compared to the diabetic control mice as demonstrated in figure 2.

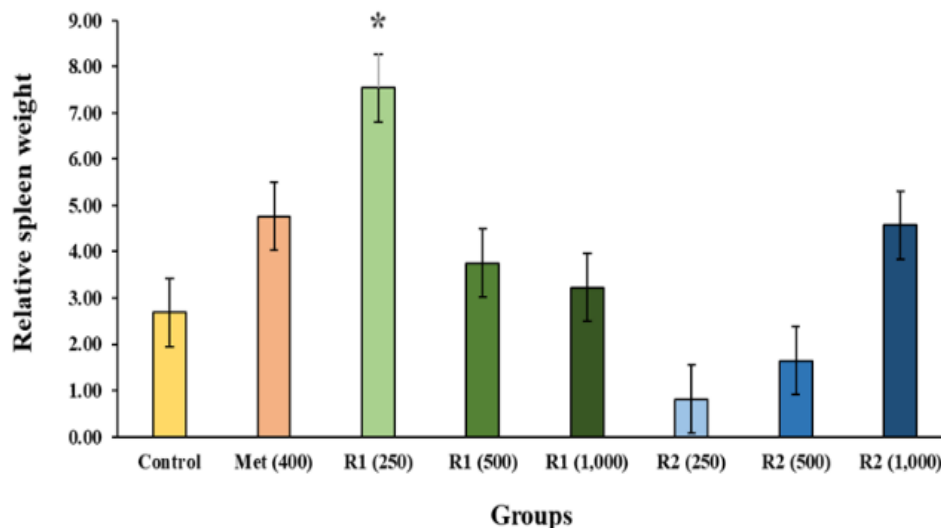


Figure 2 Relative spleen weight of diabetic mice after the administration of Thai herbal recipes for 28 days. Bar graph showed mean ± SEM (n=2-4 mice per group). *p < 0.05 vs. the control group.

Effect of Thai herbal recipes on peripheral WBC and their differentials parameter profiles in diabetic mice

The WBC count and differential in the diabetic mice are demonstrated in table 4. The treatment with herbal recipe 1 at the dose of 1,000 mg/kg significantly

increased in total WBC counts and the total number of lymphocytes and basophils in peripheral blood when compared with the diabetic control group. A significant decrease in the total number of monocytes was observed in diabetic mice treated with metformin, herbal recipe 1

at the doses of 250 and 500 mg/kg and herbal recipe 2 at the dose of 1,000 mg/kg when compared with the diabetic control mice. The total number of neutrophils

and eosinophils in diabetic mice treated with metformin and both herbal recipes were similar to the diabetic control mice.

Table 4 Peripheral WBC and their differentials parameter profiles of diabetic mice after the administration of Thai herbal recipes for 28 days. Data are displayed as mean ± SEM (n=2-4 mice per group). *p < 0.05 vs. the control group.

Group	WBC (x10 ³ /μL)	Neutrophil (x10 ³ /μL)	Lymphocyte (x10 ³ /μL)	Monocyte (x10 ³ /μL)	Eosinophil (x10 ³ /μL)	Basophil (x10 ³ /μL)
Control	2.65±0.55	1.28±0.47	0.92±0.03	0.40±0.11	0.05±0.03	0.00
Met (400)	2.04±0.93	1.64±0.97	1.00±0.39	0.18±0.07*	0.06±0.06	0.00
R1 (250)	3.08±0.45	1.27±0.51	1.62±0.38	0.12±0.05*	0.07±0.02	0.00
R1 (500)	2.47±0.69	1.59±0.47	0.65±0.19	0.18±0.06*	0.07±0.03	0.00
R1 (1,000)	6.61±2.07*	2.39±0.69	3.66±2.90*	0.46±0.08	0.09±0.07	0.02±0.02*
R2 (250)	2.97±0.92	1.48±0.70	1.15±0.12	0.24±0.03	0.10±0.07	0.00
R2 (500)	3.79±0.50	2.36±0.80	1.15±0.24	0.24±0.09	0.05±0.03	0.00
R2 (1,000)	2.07±0.72	0.68±0.13	1.32±0.70	0.05±0.01*	0.02±0.01	0.00

Discussion

Nowadays, the number of elderly people around the world is increasing [1]. Thus, the incidence of various diseases in the elderly has increased as well. Type 2 DM is one of those that has a high rate of disease in the elderly [2]. Both aging and the incidence of Type 2 DM cause progressive degeneration of various systems in the body of the elderly including the immune system. Therefore, it is important to prevent the deterioration of the immune system that occurs in the elderly. It has been confirmed that several herbs comprising *M. cochinchinensis*, *C. ternatea*, and *C. zeylanicum* have immune-enhancing effects [7-9]. Previous phytochemical studies reported the presence of carotenoids, especially lycopene and β-carotene in *M. Cochinchinensis* arils extract which these carotenoids have been confirmed to have immune-enhancing and antidiabetic properties [15-19]. Moreover, both immune-enhancing and antidiabetic activities of anthocyanins, especially ternatins, isolated from *C. Ternatea* flowers have been shown previously [20-22]. Triterpene saponins and pregnane glycosides purified from *G. Inodorum* aerial parts demonstrated antidiabetic activity, however, their immune-enhancing activity has not yet been reported [23,24]. In addition, there are reports about immunomodulatory and antidiabetic activities of proanthocyanidin and cinnamtannin B1 obtained from *C. zeylanicum* stem barks [25-28]. In addition, two Thai herbal recipes with these plants have been invented. The first herbal recipe consisted of *M. cochinchinensis*, *C. ternatea*, and *G. inodorum* while the second herbal recipe contains *C. zeylanicum* instead of

G. inodorum. The present study was undertaken to evaluate the immunomodulatory effect of these two Thai herbal recipes in diabetic models of male retired breeder mice. Antidiabetic and immunological parameters were observed in this study.

STZ, a compound derived from *Streptomyces achromogenes*, is clinically used for pancreatic β cell carcinoma treatment. Because this compound targets and damages pancreatic β cells, thereby causing subsequent hypoinsulinemia and hyperglycemia [29]. A previous study has reported that a single i.p. injection of STZ at the dose of 100 mg/kg in 8-week-old male ICR mice produced slowly progressive non-insulin-dependent diabetes mellitus (NIDDM) which increased non-fasting serum glucose levels over a 12-week study period [30]. Metformin is one of the oral antihyperglycemic agents used for the treatment of Type 2 DM. The general mechanisms of actions of metformin are the suppression of hepatic glucose production, the enhancement of glucose uptake, and the improvement of insulin sensitivity in peripheral tissues [31]. In the present study, male retired breeder mice were induced to develop diabetes by injecting 80 mg/kg STZ (i.p.) and metformin at the dose of 400 mg/kg was used as a reference drug in the diabetic positive control group.

A rise in blood glucose levels over 400 mg/dL was found in male retired breeder mice within 7 days after the STZ injection. Previous research has indicated that mouse strains with fasting blood glucose levels greater than 250 mg/dL showed common signs and symptoms of diabetes such as polyuria and polydipsia [32]. These diabetic symptoms were also observed in the present

study. After a single administration of metformin, the diabetic mice had a decrease in blood glucose level at 60 minutes after administration. It seemed that treatment with both herbal recipes could decrease blood sugar levels at 240 minutes after a single administration.

Moreover, treatment with metformin and herbal recipes showed the hypoglycemic effects in diabetic mice after repeated administration for 7 days. When comparing between the doses of herbal recipes, the low dose of both herbal recipes tended to be the most effective at lowering blood glucose levels. The hypoglycemic effect of these herbs is composed in the recipes confirmed in many studies [10-13]. Nevertheless, mice deaths occurred in all groups and the living diabetic mice had higher blood sugar levels after the 7th day of treatment, possibly as a result of higher mortality rates at older ages associated with the development of diabetes was severe in older mice. Therefore, the hypoglycemic effects of herbal recipes were dose independent.

Next, we examined the effect of two Thai herbal recipes on immunological parameters. The thymus is a primary lymphoid organ of the immune system where the process of T cell development is taking place. The reduction of functions and thymic atrophy occur with age [33]. Our study reported that the relative thymus weight appeared to increase in the diabetic mice treated with a low dose of herbal recipe 1 and a high dose of herbal recipe 2. The spleen is one of the secondary lymphoid organs of the immune system. One of the spleen's functions is involved in the initiation of innate and adaptive immune responses in which antigen-presenting cells (APCs) process antigens and present them to T lymphocytes [34]. Our study showed that the relative spleen weight was significantly increased in the diabetic mice treated with a low dose of herbal recipe 1. Meanwhile, the diabetic mice treated with herbal recipe 2 at low and middle doses seemed to decrease their relative spleen weight. The results obtained from this study demonstrated that the low dose of herbal recipe 1 and the high dose of herbal recipe 2 tended to increase the relative organs weight of thymus and spleen.

WBCs circulate in the circulatory system and function in both innate and adaptive immune responses. All granulocytes (neutrophils, eosinophils, and basophils), monocytes, and macrophages are a part of the innate immune system. Meanwhile, a vital component of the adaptive immune system is lymphocytes [35]. The present study showed that the diabetic mice treated with a high dose of herbal recipe 1 had a significant increase in total WBC, lymphocytes, and basophil counts. In addition, a significant decrease in the monocyte count was observed in the diabetic mice treated with metformin, herbal recipe 1 at low and middle doses, and herbal recipe 2 at high dose. However, a previous study demonstrated that alloxan-induced diabetic rats treated with the extract of *C. ternatea*, the component of both herbal recipes, showed a significant increase in the total number of WBCs and

lymphocytes, whereas a total number of monocytes and eosinophils exhibited a significant decrease [8]. Additionally, treatment with the *C. zeylanicum* extract, the component of herbal recipe 2, significantly increased the total number of WBCs in alloxan-induced diabetic rats [36]. The effect of herbal recipes on peripheral WBC and their differentials parameter profiles seemed to be dose independent.

Conclusion

In summary, our study shows the hypoglycemic and immunomodulatory effects of two Thai herbal recipes in diabetic aging mice. Although treatment with herbal recipes and metformin was found to reduce blood glucose levels, it could not improve body weight loss in diabetic aging mice. Treatment with herbal recipes in diabetic aging mice also caused a decrease in peripheral monocyte count. Diabetic aging mice treated with herbal recipe 1 at 250 mg/kg dose had a significant increase in relative spleen weight, whereas 1,000 mg/kg dose of herbal recipe 1 treatment significantly increased peripheral WBC, lymphocyte, and basophil counts. In addition, diabetic aging mice treated with herbal recipe 2 at 250 and 500 mg/kg seemed to decrease relative spleen weight, whereas 1,000 g/kg dose of herbal recipe 2 treated group tended to increase relative thymus weight. Altogether, herbal recipes 1 seemed to have an immune-boosting effect when administered to diabetic aging mice.

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Molecular Characterization of the Full-length Bipartite Begomovirus Causing Pepper Yellow Leaf Curl Disease, a Common Cause of Crop Damage in Northeast and Central Thailand

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ABSTRACT

Introduction: The incidence of the yellow leaf curl disease of pepper is an important and serious threat for the production of pepper in Thailand. The disease is caused by whitefly-transmitted begomoviruses which can lead to severe economic losses of the crop.

Objective: To determine and describe the complete sequences of begomovirus important species isolated from field infected pepper plants

Methods: In this study, six diseased samples of the *Capsicum annuum* plants (showing yellow mosaic and leaf curling symptom) were collected from outbreak areas during a survey in 2016 – 2019 and used to extract total DNA. The full-length viral genomes were amplified by rolling circle amplification technology (RCA), cloned and sequenced bi-directionally. The generated sequences were then assembled and characterized including their phylogenetic relationships with other selected begomoviruses.

Results: The sequences of the DNA-A genome from all six isolates showed the highest identity (97%) with the isolates of *Pepper yellow leaf curl Thailand virus* (PepYLCTHV). For the DNA-B component, it showed the closest cluster with those of PepYLCTHV (90% similarity).

Conclusion: Based on the literature review and current evidence, this is the report confirming the presence of the PepYLCTHV bipartite begomovirus from Thailand.

Keywords: *Capsicum*; *Begomovirus*; RCA; *Pepper yellow leaf curl*

Introduction

Chili peppers belong to the genus *Capsicum* of the Solanaceae family native in Central and South America presently dominating the world's hot spice trade [1-2]. Flesh of *Capsicum* can be used in the pharmaceutical/medicinal aspect all over the world. *Capsicum* has been used orally for upset stomach, toothache, poor circulation, fever, hyperlipidemia, and heart disease prevention [3]. Besides, its use has also been reported for relief of muscle spasms topically to treat pain associated with osteoarthritis, shingles, rheumatoid arthritis, post-herpetic neuralgia, trigeminal

neuralgia, diabetic neuropathy, fibromyalgia, and back pain [4-5]. *Capsicum* is rich in vitamin A, vitamin B complex, vitamin E and vitamin C. These vitamins and various antioxidants are active and set against the free radicals [6]. Pungency (hotness) of the *Capsicum* is due to the presence of capsaicin which is an active component of peppers producing an intense burning sensation when it comes into contact with the skin, eyes, or mucous membranes and which gives peppers their burning taste [7]. Capsaicin is the main constituent of capsaicinoid in *Capsicum*, followed by dihydrocapsaicin, nordihydrocapsaicin,

homodihydrocapsaicin, homocapsaicin and minerals levels which are beneficial to health [8-9]. *Capsicum* is susceptible to various pests and diseases including begomoviruses resulting in yield loss and becomes the most significant problem in the main production area [10]. Reduction of planting area has been observed every year due to cost of management and disease problems [11]. The begomovirus, a member of the family Geminiviridae, has been frequently reported causing disease in many vegetable crops, ornamental plant and weed species worldwide in terms of incidence and yield losses [12]. Depending on the genera, the viral genome comprises either a single DNA-A genome (monopartite) or containing DNA-A and DNA-B (bipartite) components [13]. The symptom of begomoviruses-infected plants is yellow mosaic, leaf curling, puckering and malformation. In severe scenarios, the yield loss of the infected plants could be up to 100 percent [14-16]. In Thailand, the highest incidence of disease caused by begomovirus in pepper was observed in the central part followed by the north and the northeastern range up to 90-100 percent [17]. The aim of this research was to investigate the molecular characterization and phylogenetic relationships of begomovirus infecting peppers in major production fields of the northeastern and central regions of Thailand. The updated information regarding the diversity of begomoviruses obtained from this study is expected to be useful for planning appropriate strategies in viral disease control.

Methodology

Plant materials and DNA extraction

Six infected pepper leaf samples were collected from fields in Ratchaburi, Kanchanaburi and Khon Kaen. Total genomic DNA was prepared from the diseased leaf samples using a procedure described by Dellaporta [18].

RCA and cloning of full-length PepYLCHV segmented DNA-A and DNA-B

Full-length begomovirus genomes (DNA-A and DNA-B) were amplified from the extracted DNA using rolling circle amplification (RCA) based TempliPhi™ DNA amplification kit (GE Healthcare, USA) as instructed by the manufacturer's protocol. The reaction was undertaken by transferring 0.5 µl (10-20 ng) of template DNA to a 5 µl sample buffer. The amplification reaction used was denaturation at 95°C of template DNA for 3 minutes, and then cooled down to room temperature or 4°C. To this mixture, 5 µl of reaction buffer and 0.2 µl of enzyme mix were added, and the reaction was run for 4-18 hours at 30°C. The reaction was then terminated by incubating the mixture at 65°C for 10 minutes, and then cooled down to 4°C. The

product of the TempliPhi reaction was monomerized by restriction digestion with *BamHI*, *EcoRI*, *HindIII*, *PstI*, *Sall* and *XbaI* (Thermo Fisher Scientific, USA) for DNA-A and DNA-B. Digested products were resolved on 1% agarose gel electrophoresis and the bands with the size of ~2.8 kbp were purified using GeneJET gel extraction kit (Thermo Fisher Scientific, USA). The components were cloned into the respective site of the pUC19 vector to produce the recombinant plasmids.

Sequencing and bioinformatic analysis

Monomeric full-length clones were purified using GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, USA) using the manufacturer's protocol. The DNA samples were then sent to the 1st BASE Laboratories (Malaysia) for DNA sequencing. The recombinant clone was bidirectionally sequenced using M13 primers (M13 Forward-GTTTTCCCAGTCACGAC; M13 Reverse-CAGGAAACAGCTATGAC). Primer walking technique using the newly generated sequences (AV 5'-CCATCTGCAGGCCACAT-3'; AC 5'-AATACTGCAGGCTT-3' for segment DNA-A and 10-1F 5'-GGGACCAATTGGCAATTGCC-3'; 10-1R 5'-ACGGTTGCCAATCCTCCTCCC-3' for DNA-B) was also carried out to complete the gap of the begomovirus genomes. The DNA sequence data of each isolate were manually assembled and edited using the SeqMan program (Lasergene, USA). Multiple sequence alignments were then aligned with these closely related begomovirus DNA sequences and phylogenetic trees were generated by MEGA software version X with 1,000 bootstrap replications [19].

Results

Genome amplification and cloning of circular DNAs

The complete genome of PepYLCHV isolate was amplified from the infected pepper leaves samples as circular DNA. Restriction of RCA amplicons with *PstI* (DNA-A) and *BamHI* (DNA-B) generated a DNA fragment of ~2.8 kb product by cleaving the sequence at one position. These restricted fragments were then cloned into PUC-19 vectors. In contrast, there were two restricted DNA fragments when using *EcoRI*, *HindIII*, *Sall* and *XbaI* indicating multi-recognition sites (data not shown). Seven full-length amplicons were then cloned and sequenced. Based on the Blast analysis the DNA sequences showed highest similarity with PepYLCHV segment DNA-A (97% similarity) and with PepYLCHV segment DNA-B (90% similarity). The DNA sequences obtained were designated as PepYLCHV-KKN601, PepYLCHV-KKN603, PepYLCHV-KKN604, PepYLCHV-KRI155, PepYLCHV-KRI157, PepYLCHV-RBI-24, DNA-A isolates and PepYLCHV-KKN601 DNA-B isolate (table 1).

Table 1 List of begomovirus complete sequences used for phylogenetic analysis

Begomovirus species (isolate)	Host	Location	Abbreviation	Type	Reference length	GenBank Accession no.
<i>Eggplant golden mosaic virus</i>	<i>Solanum melongena</i>	Thailand	EGMV	DNA-A	2742	KU569586
<i>Pepper golden mosaic virus</i>	<i>Cucumis sativus</i>	Mexico	PepGMV	DNA-B	2597	MN013411
<i>Pepper huasteco yellow vein virus</i>	<i>Capsicum annuum</i>	Mexico	PHYVV	DNA-B	2582	LN848927
<i>Pepper huasteco yellow vein virus</i>	<i>Capsicum annuum</i>	Mexico	PHYVV	DNA-B	2596	MG582069
<i>Pepper leaf curl virus</i>	<i>Capsicum annuum</i>	Malaysia: Klang	PepLCV	DNA-A	2739	AF414287
<i>Pepper leaf curl virus</i>	<i>Capsicum annuum</i>	Thailand	PepLCV	DNA-A	2744	NC000882
<i>Pepper yellow leaf curl Indonesia virus</i>	<i>Pseuderanthemum reticulatum</i>	Indonesia	PepYLCIV	DNA-B	2739	MN094865
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-A	2742	KT322143
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-A	2742	KT322146
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-B	2732	MK946435
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-A	2742	MK946436
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-A	2742	NC028989
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Bemisia tabaci</i>	Thailand: Suphanburi	PepYLCTHV	DNA-B	2732	NC040186
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-B	2731	KX885224
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Thailand	PepYLCTHV	DNA-B	2730	KX885225
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Bemisia tabaci</i>	Thailand: Suphanburi	PepYLCTHV	DNA-A	2742	KX943290
<i>Tomato yellow leaf curl Kancharaburi virus</i>	<i>Solanum lycopersicum</i>	Viet Nam	TYLCKaV	DNA-B	2740	DQ169055
<i>Tomato yellow leaf curl Kancharaburi virus</i>	<i>Solanum lycopersicum</i>	Thailand: Kancharaburi	TYLCKaV	DNA-B	2752	NC005811
<i>Tomato yellow leaf curl Thailand virus</i>	<i>Solanum lycopersicum</i>	Thailand	TYLCThV	DNA-B	2737	NC000870
Begomovirus identified in This study						
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen- Thailand	PepYLCTHV	DNA-A	2733	MW715819
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen - Thailand	PepYLCTHV	DNA-B	2745	MW715820
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen- Thailand	PepYLCTHV	DNA-A	2733	MW715821
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Khon Kaen - Thailand	PepYLCTHV	DNA-A	2733	MW715822
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Ratchaburi- Thailand	PepYLCTHV	DNA-A	2742	MW715823
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Kanchanaburi- Thailand	PepYLCTHV	DNA-A	2742	MW715824
<i>Pepper yellow leaf curl Thailand virus</i>	<i>Capsicum annuum</i>	Kanchanaburi- Thailand	PepYLCTHV	DNA-A	2742	MW715825
Out group						
<i>Chili vinal mottle virus</i>						NC005778

Diversity and phylogenetic analysis

The generated nucleotide sequences were used for genetic diversity and phylogenetic relationship analysis with the sequences of selected begomoviruses by using MEGA X software. As shown in figure 1, a phylogenetic analysis of DNA-A was constructed, and it revealed that all DNA sequences obtained from this study were grouped with PepYLCTHV two sub-clades comprising the isolates identified in this study. The isolates of DNA-A from PepYLCTHV-KKN601, PepYLCTHV-KKN603 and PepYLCTHV-KKN604 grouped in a well-supported clade (bootstrap 100%). The other 3 isolates PepYLCTHV- RBI24, PepYLCTHV- KRI155 and PepYLCTHV- KRI157 were placed in the different clade containing EGMV isolate TH12WE12 (KU569586), PepYLCTHV isolate BRM103, WF-SPN-Pep2015 and SPN-PG1 (MK946436, KX943290 and KT322143). Within the DNA-B clade, the isolates PepYLCTHV-KKN601 identified in this study grouped with a clade comprising PepYLCTHV isolate BRM103 (MK946435), KON-KG5B (KX885224), WF-SPN-Pep2015 (NC040186) and TMK-KR8B (KX885225) in a well-supported clade (bootstrap 100%) and distance from *Pepper yellow leaf curl Indonesia virus* (PepYLCIV, MN094865) reported from Indonesia (figure 2).

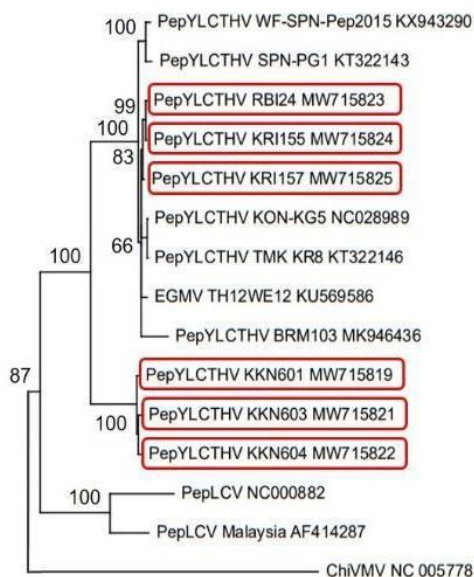


Figure 1 Phylogenetic trees constructed from aligned complete nucleotide sequences of PepYLCTHV DNA-A obtained from this study (red boxes) with other begomoviruses using the neighbor-joining algorithm. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values are numbered along branches

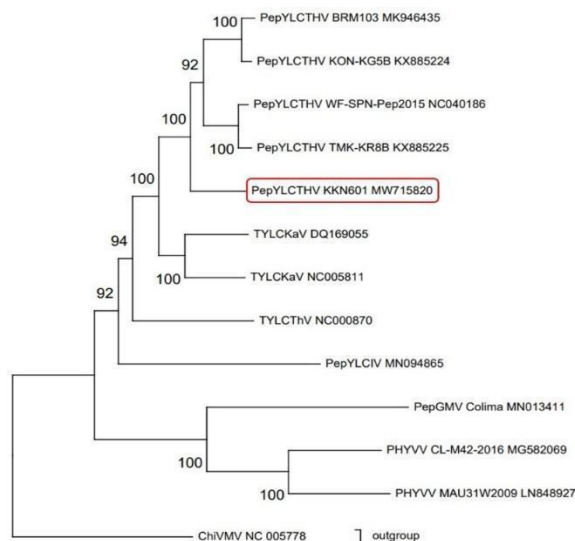


Figure 2 Phylogenetic trees constructed from aligned complete nucleotide sequences of PepYLCTHV DNA-A obtained from this study (red boxes) with other begomoviruses using the neighbor-joining algorithm. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values are numbered along branches

Discussion

Begomoviruses can infect a wide range of cultivated crops and huge economic losses worldwide [20-21]. The epidemiological surveys of viral disease in pepper major production areas in Thailand from recent studies demonstrate that the evidence of begomovirus on various varieties of pepper has gradually been increased consistently throughout the country after the first report in 2018 [17,22]. In this study, full genome sequences of DNA-A and DNA-B were amplified from field infected pepper samples. Based on sequence analyses, begomovirus isolates obtained from this study showed genome-wide pairwise identities above the 91% taxonomic threshold for inclusion in the same species [23]. This information concluded that the virus identified from this study is PepYLCTHV bipartite begomovirus and all isolates were suggested to originate from Thailand.

Conclusion

This information concluded that the virus identified from this study is PepYLCTHV bipartite begomovirus and all isolates were suggested to originate from Thailand. The generated information was obtained from full-length genome amplification of DNA-A and DNA-B from field surveyed samples. This information regarding the knowledge of the distribution and diversity of interesting relationships between the genus Begomovirus in different locations is very crucial for developing and deploying suitable resistant cultivars for pepper crop and strategies for the development of

effective integrated disease management against viruses.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Author contributions

AL carried out all the experiments, interpreted data, and prepared the first draft. SN suggested the revised manuscript, SM assisted with the DNA sequence analysis, EC reviewed and edited the manuscript.

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Therapy of strengthening spleen and Nourishing Kidney Massage in the Treatment of Global Developmental Delay

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ABSTRACT

Objective: To investigate the clinical research plan of the therapy of strengthening spleen and nourishing kidney massage in the treatment of children with global developmental delay (GDD).

Methods: A total of 60 children with GDD were randomly divided into the treatment group and the control group, 30 children of each group. Both groups of children accepted routine rehabilitation and the children were treated with Strengthening Spleen and Nourishing Kidney Massage in the treatment group plus the above treatment. The total course of two groups was 12 weeks. Before and after the treatment, children in both groups were evaluated with the Gesell Development Scale (GDS), Gross Motor Function Measure-88 (GMFM-88) and Infant-Junior Middle School Students Social life Abilities Scale (S-M scale). And the safety of the treatment was evaluated.

Conclusion: Pediatric Massage, as a non-pharmaceutical therapy, provides a new idea for the treatment of GDD.

Keywords: strengthening spleen and nourishing kidney; massage therapy; global developmental delay;

Introduction

全面性发育迟缓 (global developmental delay, GDD)是指发育早期的儿童存在2个或2个以上发育指标/里程碑明显延迟,包括粗大运动和精细动作、认知、语言理解和表达、个人和社会发展以及日常生活等多个领域 [1]。GDD是婴幼儿时期一种常见的早发性慢性神经系统疾病 [2],属于一种过渡性诊断,发病率在3%左右 [3]。患者多在5岁以后发展成智力障碍,且情绪行为异常率显著增加,故该病严重威胁患儿的生活质量,危害身心健康,并可导致终身残疾 [4]。

GDD在中医学中并无专有病名,但根据其临床表现,该疾病可归属于中医“五迟”“五软”“痿证”“胎怯”“胎弱”等范畴,病因病机复杂,但大都与先天不足及后天失养有关。而“脾为后天之本”“肾为先天之本”,脾肾两脏与该病的发生发展密切相

关,故通过健脾益肾改善脾肾功能在治疗中必不可少。小儿推拿作为一种非药物疗法,广泛运用于中医儿科临床,这也为GDD的治疗提供了另一种新思路。故针对健脾益肾推拿法在治疗GDD中的运用,提出以下临床研究方案。

Methodology

1. 研究对象

选取符合GDD诊断标准患儿60例,采用随机化方法。通过SAS统计分析系统产生60例患儿所接受处理的随机安排,列出流水号01~60所对应的治疗分配,治疗组30例,对照组30例。

1.1 纳入标准: (1)符合GDD诊断标准者; (2)符合中医辨证属脾肾两虚型; (3)受试患儿监护人签署知情同意。

1.2 排除标准：(1) 已诊断为遗传性代谢性疾病、染色体疾病的全面性发育迟缓患儿；(2) 明确诊断为脑性瘫痪、孤独症谱系障碍、发育协调障碍等其他神经发育障碍性疾病；(3) 癫痫发作，发育倒退、颅内占位性病变、脑积水或其他进展性疾病；(4) 合并急慢性传染性疾病。

1.3 中止标准：(1) 治疗期间出现严重并发症或者合并症者；(2) 出现严重不良反应者；(3) 未按规定定期接受治疗、定期评估，无法判定疗效者。

1.4 脱落标准：(1) 治疗及观察过程中自然脱落、失访或死亡者；(2) 发生不良反应或者并发症而退出实验者；(3) 依从性差，治疗疗程不够者。

2. 诊断标准

2.1 小儿全面性发育迟缓的西医诊断标准

参照 2013 版《美国精神疾病诊断与统计手册》(DSM-5) 中 GDD 的诊断标准拟定：(1) 5 岁以下发育早期的儿童；(2) 有 2 个或 2 个以上的发育里程碑的显著延迟；(3) 年龄过小不能完成一个标准化智力功能的系统性测试，不能确切的评估病情的严重等级。

2.2 发育迟缓程度分级

以 Gesell 发育诊断量表发育商测评结果判断，当 $55 \leq DQ \leq 75$ 为轻度发育迟缓， $40 \leq DQ \leq 54$ 为中度发育迟缓， $25 \leq DQ \leq 39$ 为重度发育迟缓， $DQ < 25$ 为极重度发育迟缓。

2.3 中医辨证分型标准

参照《实用中医儿科学》、《中医儿科学》中有关五迟五软、智能发育迟缓及胎怯的论述，结合中医对小儿发育迟缓辨证论治的临床经验，拟定小儿全面性发育迟缓脾肾两虚证型的诊断标准：

主症：(1) 头项萎软，不能抬举；(2) 柱骨软弱，腰脊无力，坐立不稳；(3) 手软腕垂，不能握举；(4) 足软弛缓，站立不能；(5) 口软唇薄，咀嚼困难；(6) 言语不清，智能低下；(7) 肌肉松弛，活动无力；(8) 疲倦喜卧，多卧少动；(9) 面色不华；(10) 脉沉弱或细弱。

次症：(1) 头发稀疏萎黄；(2) 牙齿生长迟缓，生而牙质不好；(3) 视物不清；(4) 凶门宽大；(5) 食欲不佳；(6) 口角流涎；(7) 周身羸瘦；(8) 自汗盗汗；(9) 神情呆滞；(10) 舌质淡，苔薄白。

凡具备上述证型标准中 3 项主症，或 2 项主症加 2 项次症，或 1 项主症加 4 项次症者，即可纳入脾肾两虚证型。

3. 治疗方法

两组均采用康复训练。康复训练由固定的专业的康复治疗师对患儿进行综合康复训练，综合儿童各阶段神经发育心理特点和个体发育异常情况，根

据每一位患儿的情况制定个体化具体的康复治疗方

案，包括物理因子治疗、运动疗法、作业疗法、语言认知训练、感觉统合训练、引导式教育等。康复训练每次治疗 45~60 分钟，每周 5 次，以 20 次为 1 疗程，共连续治疗 3 个疗程。

治疗组在康复训练的基础上行健脾益肾推拿法。基础方：揉百会 100~300 次，揉四神聪 100~300 次，补脾经 100~300 次，补肾经 100~300 次，推三关 100~300 次，摩腹 3~5 分钟，摩气海 2 分钟，摩关元 2 分钟，按揉足三里 100~300 次，按揉太溪 100~300 次，捏脊 4 遍，按揉脊柱及脊旁各穴（以脾俞、肾俞、命门为主）共 3~5 分钟，横擦肾俞、命门，以透热为度。时间约 15 分钟。

循经推拿：沿手阳明大肠经、足阳明胃经循行施以一指禅指揉、拿揉或弹拨等，按揉肩髃、臂臑、曲池、手三里、合谷、髀关、伏兔、梁丘、丰隆、解溪等腧穴，由轻到重做内旋外旋肩关节、外展外旋髋关节、屈曲膝关节、背曲踝关节等，操作 5~7 遍。时间约 10 分钟。

辨症取穴：因本病症情复杂，具有不同发育指标及发育程度的不同延迟，根据各项指标发育程度及临床症状不同，配予不同腧穴及推拿手法，以认知障碍为主，加按揉顶颞前斜线、顶颞后斜线，拿五经等手法醒神开窍；以语言障碍为主或伴有流涎等，加按揉哑门、廉泉、地仓、颊车等手法促进发音，减少流涎；合并听觉障碍者，加按揉听宫、听会、耳门等手法以聪耳开窍；合并视觉障碍者，加按揉精明、鱼腰、四白等手法疏通眼部气血，改善视力；合并癫痫者，加清心经、清肝经、拿风池等手法泻肝息风；合并先天性心脏病者，加按揉膻中、内关、心俞等手法调理心胸气血（以上选穴均为 100 次）。时间约 5 分钟。

以上推拿操作皆在温暖室温下进行，以婴幼儿爽身粉作为推拿介质。患儿处于平静状态，无过饥过饱，着宽松柔软衣物，排空小便，医者保持平心静气、精神内守。以上推拿操作次数及时间以 3-5 岁 GDD 患儿为准，2-3 岁 GDD 患儿取之三分之二，小于 2 岁 GDD 患儿操作次数取之三分之一，并以轻松柔和为总的操作原则。

推拿治疗每次约 30 分钟，隔天一次，每周 3 次，每 12 次为 1 疗程，共连续治疗 3 个疗程。

4. 疗效观察

主要临床疗效指标为 Gesell 发育量表中文修订版、粗大运动功能评估量表 (GMFM-88) 及婴儿-初中生社会生活力量表 (S-M)。

4.1 Gesell 发育量表

Gesell 发育量表中文修订版包括以下 5 项指标：大运动 (GM)、精细运动 (FM)、语言 (L)、适应性 (AD) 以及个人社交 (PS)。以发育商 (development quotient, DQ) 作为评估发育迟缓程

度的标准, $DQ = (\text{发育年龄}/\text{实际年龄}) \times 100$ 。
 $DQ \geq 86$ 为正常, 55~75 为轻度迟缓, 40~54 为中度迟缓, 25~39 为重度迟缓, < 25 为极重度迟缓[5]。两组患儿分别于治疗前后进行 Gesell 发育量表评分比较, 以 DQ 提高水平评定治疗效果。

4.2 粗大运动功能评估量表 (GMFM-88)

粗大运动功能评估量表 (GMFM-88) 包含 88 个项目, 4 级评分, 每项 2 分, 分为 A、B、C、D、E 5 个维度, 其中 A 维度评定翻身和卧位, B 维度评定坐位, C 维度评定跪和爬行, D 维度评定站, E 维度评定跳和走跑 [6]。两组患儿分别于治疗前后按照其他完成制定动作程度评分, 以五个区的总分评估患儿的整体粗大运动功能。评分越高表示患儿粗大运动功能越好。

4.3 婴儿-初中生社会生活能力量表 (S-M)

婴儿-初中生社会生活能力量表 (S-M) [7] 评定内容为 6 个方面能力测评, 包括独立生活、运动、作业、交往、参加集体活动和自我管理, 以测定原始分后结合实际年龄换算为标准分, 社会生活能力评定等级: 正常或正常以上: 标准分 ≥ 10 分; 边缘水平: 标准分 9 分; 轻度异常: 标准分 8 分; 中度异常: 标准分 7 分; 重度异常: 标准分 6 分; 极重度异常: 标准分 ≤ 5 分。

4.4 不良事件

观测内容包括推拿过程中出现的各种不良反应, 如骨折、骨与关节损伤、软组织损伤、脱臼、晕厥、出血、脏器损伤、剧烈疼痛等。

5. 疗效及安全性评价标准

5.1 疗效判定标准

(1) Gesell 发育量表中文修订版中 GM、FM、L、AD、PS 五个能区的发育商及总发育商; (2) 粗大运动功能评估量表 (GMFM-88) 总分; (3) 婴儿-初中生社会生活能力量表 (S-M) 评分。

5.2 安全性评价标准

1 级: 安全, 不存在任何不良反应; 2 级: 比较安全, 若有不良反应, 无需任何处理, 可继续治疗; 3 级: 有安全性问题, 存在中等程度的不良反应, 处理后仍可继续治疗; 4 级: 因不良反应而中止治疗。

6. 统计方法

数据录入 Excel 表建立数据库, 采用 SPSS19.0 软件统计分析。计数资料使用卡方检验 (χ^2), 计量资料数据以平均数 \pm 标准差 ($\bar{x} \pm S$) 表示。检验水准 $\alpha = 0.05$, 以 $P < 0.05$ 为差异有统计学意义。每组的同一时间点的组间比较如治疗前和治疗后, 采用独立样本 t 检验; 每组不同时间点如治疗前后差异的比较, 采用配对样本 t 检验。

数据录入 Excel 表建立数据库, 采用 SPSS19.0 软件统计分析。计数资料使用卡方检验 (χ^2), 计量资料数据以平均数 \pm 标准差 ($\bar{x} \pm S$) 表示。检验

水准 $\alpha = 0.05$, 以 $P < 0.05$ 为差异有统计学意义。每组的同一时间点的组间比较如治疗前和治疗后, 采用独立样本 t 检验; 每组不同时间点如治疗前后差异的比较, 采用配对样本 t 检验。

Discussion

小儿推拿具有平衡阴阳、调理脏腑、补益气血、舒经通络的功效。诸多临床文献 [5-9] 指出, 小儿推拿不仅能够有效促进患儿运动、智力、语言、社会交往等多方面能力的发展, 还能够提高儿童体质 [10], 降低发病率, 循证医学也证实小儿推拿是一种促进儿童生长发育的有效且安全的疗法 [11]。健脾益肾推拿法在治疗 GDD 时于全身各部均选取穴位。

头部穴位选取百会穴与四神聪。百会穴在巅顶, 为“三阳五会”之所, 内通于脑, 而“脑为髓之海, 其输上在于其盖 (百会)”, 这阐述了脑与百会穴的密切联系。而四神聪, 在“百会四面各相去同身寸一寸”, 亦在巅顶, 前后两穴位于督脉, 左右两穴临近足太阳膀胱经, 故其为阳气聚集之所, 常与百会穴合用, 有振奋阳气、安神益智、开窍醒神的功效。现代研究表明, 头部推拿能够加速脑损伤修复, 改善大脑功能 [12]。并且, 百会穴与四神聪都为治疗脑病的重要穴位, 有增强脑供血、改善脑部微循环、促进修复脑细胞和神经、减少炎性递质、提高认知水平和运动能力的作用 [13,14]。该病的双侧穴位选取脾经、肾经及三关穴。“小儿百脉会于两掌”, 所以在小儿推拿中注重对于上肢穴位的使用。脾经, 又名脾土。脾为后天之本, 主运化, 主四肢肌肉。而小儿脾常不足, 故“大指脾土, 宜多补”, 补脾经效用“似人参、白术”, 有健脾主运、补益气血以及健运四肢的作用。肾经, 又名肾水。肾为先天之本, 藏精, 主骨生髓, 与生长发育有关。补肾经能促进 GDD 患儿的生长发育, 古人谓“小指补肾, 焉差杜仲地黄”, 后世称“肾穴通脑最快”, 能促进患儿语言、智力等方面的发展。脾经与肾经穴的配伍又能在增强各自穴位治疗作用的基础上, 扩大相应治疗范围。故多用补脾经、补肾经治疗先后天不足。三关穴, 在前臂桡侧, 自腕横纹至肘横纹, 为一直线。该穴位性热, 故“推上三关为热为补”, 可以“代却麻黄肉桂”, 能够温补下元, 和血顺气, 培养一身之根本。并且, 三关穴与肾经穴常作为对穴使用, 可增强肾经的治疗作用 [15]。

下肢部选用足三里与太溪。足阳明经多气多血, 足三里为足阳明胃经的合穴, 又是胃的下合穴, 所谓“合治内腑”, 通过按揉足三里既可以达到促进气血生成, 又可和降气机。而脾胃互为表里, 足三里与补脾经配合可使气机升降相依, 同养后天

之气，补虚强壮。同时补脾经、肾经以及按揉足三里等手法能够促进儿童对于营养物质的吸收，对包含语言、运动社交等方面的身心发育有着积极地作用 [16,17]。太溪穴，为足少阴肾经的原穴，所谓“五脏有疾，当取之十二原”，故其具有补肾气、填肾精的作用。与补肾经配合使用，可加强调补先天的功效。现代研究表明，刺激太溪穴，可以激活额内侧回 (BA6) [18]，此区域与记忆、语言及学习模仿功能相关，是“肾为作强之官，伎巧出焉”的体现 [19]。同时，足三里与太溪合用，亦有健脾益肾，培补先后天的作用。

在腹部选用关元、气海以及小儿推拿中常用的特定穴——腹。关元穴是足三阴经与任脉交会之处，内应胞宫精室，有补益精血、培肾固本的功效。气海，为“元气之海”，与关元同属于任脉，为人体元气生发之地，能主一身之气。按揉气海穴，能补益先后天之本，达到益肾固精、培元固本的作用，从而改善患儿脏器疲惫的状态。腹作为小儿推拿的特定穴，该穴定位于整个腹部。《厘正按摩要术》言：“胸腹者...阴阳气血之发源。”常用摩法，以脐为中心，在其周围循环反复操作。这三个穴位的配伍，体现了小儿推拿“点面结合”的特点，可作用于肠道改善肠道菌群，并通过调节脾脏与胸腺，增强患儿免疫力，其后通过调控脑内神经递质以活跃不同脑功能区，从而改善患儿认知、记忆、交流等功能 [20]。并且，通过调节交感及副交感神经，增强胃肠蠕动，促进消化液分泌，增强消化吸收功能 [21]，提升患儿体质。同时，腹肌是躯干与四肢的连接部位，通过腹部推拿可以有效改善患儿脏腑整体功能，提高患儿的运动能力及社会适应能力，改善患儿的生活质量 [22,23]。

在背部，选取脾俞、肾俞、命门、脊等穴位。脾俞、肾俞为背俞穴，与五脏六腑相关联，不仅能反应脏腑功能状态，也能对其状态进行调整。脾俞、肾俞配合补脾经、补肾经，可以增强健脾益肾的作用。命门，为督脉要穴，“命门者，诸神精之所舍，原气之所系也”，在两肾之间，为肾间动气，在操作中与肾俞配合，可发挥补肾固精、壮骨柔筋之功效。脊，为小儿推拿特定穴，位于背后正中，为督脉所行之处，常用手法为捏脊。施术部位在督脉、两侧夹脊穴及膀胱经，有调阴阳、理气血、和脏腑、培元气的作用 [24]，在脑瘫幼鼠的研究中表明，捏脊可以促进幼鼠的生长发育 [25]。临床研究表明，捏脊可以通过调节脑瘫患儿体内微量元素吸收，促进神经、运动系统的发育 [26]，可明显改善婴儿期患儿的运动发育迟缓 [27]。并且，体表的机械刺激可增强脑神经细胞发育速度，减少或避免部分迟发性神经细胞的凋亡 [28]。

Conclusion

综上所述，中医理论认为，GDD的病机为脾肾不足，气血亏虚，精髓不充。故此，健脾益肾是该疾病治疗中的主要原则。健脾益肾推拿法，对于体表穴位的刺激，可以增强脾肾两脏的功能，调补气血、填精固髓，使筋骨强健、脑髓得养，从而促进儿童生长发育，提高患儿的生存质量。小儿推拿是临床常用的非药物治疗手段，具有安全有效的治疗优势。该方案的提出，不仅为推拿治疗 GDD 提供了理论依据，也为该病的治疗提供了新的思路及可行方案。

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Study on the mechanism of exercise therapy in promoting neural stem cell mobilization after stroke

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ABSTRACT

Introduction: Stroke has become the third leading cause of death in humans, most of which are ischemic strokes. Exercise therapy is currently very popular and loved by most people. Studies have found that exercise training can promote neurogenesis, angiogenesis, synaptic plasticity, and neural stem cell proliferation and differentiation after cerebral ischemia. The Wnt/ β -catenin signaling pathway regulates cell cycle and neurogenesis.

Objective: To observe the mobilization of endogenous neural stem cells by exercise therapy in rats with cerebral ischemia and its mechanism.

Methods: Thirty SPF adult SD rats were selected, 8 rats were randomly selected as the sham operation group (sham), and the remaining rats were constructed as a middle cerebral artery occlusion (MCAO) model, and then the successfully modeled rats were randomly divided into the model group (MCAO) and exercise group (RUN), exercise group was given exercise treatment 24 hours after MCAO operation, once a day for 9 consecutive days, and model group and sham operation group were not treated. The neurological deficits of the rats were detected by behavior, the volume of cerebral infarction was detected by TTC staining, and the expressions of DCX and Wnt/ β -catenin pathway-related proteins wnt3a and β -catenin in the infarcted cortex were detected by western blot.

Results: Compared with the sham operation group, the mNSS score of the model group was significantly higher ($P < 0.001$), and compared with the model group, the mNSS score of the exercise group was lower ($P < 0.001$). The Fault score was lower ($P < 0.001$), and compared with the model group, the Foot-Fault score of the exercise group was higher ($P < 0.01$); TTC staining showed that the cerebral infarction volume in the sham operation group was 0. The volume of cerebral infarction in the group increased significantly ($P < 0.001$). Compared with the model group, the volume of cerebral infarction in the exercise group was reduced, but the difference was not statistically significant. Western blot showed that compared with the sham operation group, the protein expressions of wnt3a ($P < 0.01$) and β -catenin ($P < 0.05$) in the model group were lower, and compared with the model group, the protein expressions of DCX ($P < 0.05$) and wnt3a ($P < 0.01$) in the exercise group were higher.

Conclusion: Exercise therapy may promote the mobilization of endogenous neural stem cells in rats with cerebral ischemia by activating wnt/ β -catenin.

Keywords: exercise; cerebral ischemia; wnt/ β -catenin; neural stem cells; mobilization

Introduction

脑卒中又称脑中风，是脑部血管堵塞或破裂导致的急性脑血管病。脑卒中分为缺血性卒中和出血性卒中，缺血性卒中约占所有卒中的 69.6%，并且大多为大脑中动脉闭塞所致[1]。脑卒中具有较高的发病率和死亡率，已成为我国农村第二位和城市第三位死亡原因[2]。尽管多年来人们对脑卒中的病

因、病机等有了较深的认识，但还是缺乏较为有效且能阐明其作用机制的治疗措施。

神经干细胞是能自我更新并多向分化为神经元和胶质细胞的细胞群，其主要位于侧脑室室管膜下区 (SVZ) 和海马齿状回的颗粒下层 (SGZ) [3]。一般情况下神经干细胞处于静息状态，脑缺血后，

存在于 SVZ 的神经干细胞增殖, 迁移至缺血损伤的纹状体, 分化为神经元, 新生神经元部分替代坏死神经元, 从而修复神经功能[4]。存在于 SGZ 的神经干细胞被激活后增殖, 大多数增殖的神经干细胞可以分化成神经元, 随后, 增殖的神经干细胞会迁移至颗粒细胞层, 进而分化为神经元和神经胶质细胞, 新的神经元可以参与海马神经元的再生[5,6]。研究发现, 脑卒中后利用外源性神经干细胞移植也能最大限度恢复缺失的神经功能, 但面临伦理、肿瘤、免疫排斥等问题而遭到限制[7,8]。因此, 尽可能令脑卒中后神经干细胞增殖、迁移并分化为特定的功能性细胞是我们目前需要解决的难题。

调查研究发现脑卒中高危人群危险因素占比前三项分别为高血压、血脂异常和运动缺乏[2]。运动疗法是脑卒中后康复治疗中的重要组成部分, 其可促进神经发生、血管生成、增强树突修饰和突触可塑性^[9,10]。近来研究表明, 运动训练改善脑卒中后的神经功能与促进内源性神经干细胞迁移有关[11]。运动训练可以上调神经干细胞的数量, 有促进干细胞分化为神经元的潜能[12], 促进大鼠梗死周围运动皮层中新的树突和突触形成[13], 但其作用机制不太明确。

经典 Wnt/ β -catenin 信号通路在调节成人神经发生中发挥关键作用[14], 其与中枢神经系统密切相关, 参与神经干细胞增殖和分化的调节[15]。 β -catenin 在 Wnt/ β -catenin 信号通路中占据重要地位, Wnt 途径通过调节降解复合物来调节 β -catenin 的稳定性和最终的基因表达。Wnt3a 是 Wnt/ β -catenin 信号通路中的一个重要蛋白, 其分布广泛, 普遍认为其能激活 Wnt/ β -catenin 信号通路。因此, 本文研究运动治疗是否通过激活 Wnt/ β -catenin 信号通路从而促进脑卒中后神经干细胞动员。

Methodology

1. 试剂

兔抗 DCX 购自 abcam, 兔抗 β -catenin 购自 abcam, 山羊抗兔二抗购自 abcam, β -actin 购自索莱宝, TTC 粉末购自索莱宝, BCA 蛋白浓度试剂盒购自碧云天公司。

模型建造及分组

SPF 级雄性 SD 大鼠 30 只(180-220 g), 购自湖南斯莱克景达实验动物有限公司, 分笼饲养, 5 只 1 笼。置于 08:00-20:00 光照、20:00-08:00 黑暗的实验室中, 昼夜比为自然光控节律。室温控制于 22-26°C, 室内相对湿度 40-70%。适应性喂养大鼠 1 周左右, 待其体重增加至 250-280 g 左右开始进行大脑中动脉闭塞(MCAO)模型建造。按随机数字法将大鼠分为假手术组(sham)、模型组(MCAO)、运动组(RUN)。MCAO 造模: 腹腔注射 10% 水合氯醛进行麻醉, 仰卧位平躺在木板上, 剪去颈部正中皮肤, 撑开两边肌肉组织暴露视眼, 眼科细镊钝性分离并

活结结扎颈总动脉, 分离颈外动脉在远端死结结扎, 颈外动脉近端系根细线, 颈内动脉活结结扎, 用眼科剪于颈外动脉近心端剪一小口, 插入直径为 0.4-0.42 mm 的硅胶头线栓, 使其沿颈外动脉进入颈内动脉, 后稍系紧预备丝线, 解开颈内动脉, 将栓线从颈内动脉插入至大脑中动脉处(线栓顶端有阻塞感停止, 深度约 18-20mm), 立即系紧预备丝线, 开始计时。随后松开颈总动脉, 并缝合切口。60 min 后, 完全拔出线栓, 待大鼠清醒后放回笼中喂养。假手术组的动物除了未插栓线外, 其余手术步骤与手术组相同。

2. 治疗

MCAO 造模后 24 h, 将造模成功的大鼠随机分为模型组和运动组。MCAO 手术前两天, 对所有大鼠进行适应性训练, 10min/day, 5-8m/min, 剔除不会跑步运动大鼠, 将适应良好的大鼠编组进行后续实验。运动组大鼠在 MCAO 造模后 24 对其进行跑台运动, 1 次/天, 30min/天, 持续 9 天。模型组和假手术组正常饲养, 不作其它干预处理。

3. 神经功能缺失评分

大鼠 9 天治疗结束后, 采用 7 分制神经功能缺失评分对大鼠神经功能进行评定。

4. Foot-Fault 评分

使大鼠通过 100 cm 长的水平梯(每梯间隔 2 cm), 用于反应大鼠肢体功能缺损和运动协调能力。

5. TTC 染色

麻醉处死大鼠, 将新鲜的大脑组织切为 2mm 厚, 用 2%TTC 染液浸没切片, 37°C 避光孵育 20min 左右。

6. western blot

9 天治疗结束后, 麻醉处死大鼠, 断头取脑, 取适量梗死灶周围皮层组织, 加入裂解液裂解。用 BCA 试剂盒进行蛋白定量、配制 SDS-PAGE 胶、电泳、转膜、5%脱脂奶粉封闭 2h、TBST 洗膜、孵一抗 1 h, 兔抗 DCX(1:1000)、兔抗 β -catenin(1:400)、兔抗 Wnt3a (1:1000) 过夜, 次日孵育二抗 1 h(1:3000), 洗膜、显影。

Results

1. 神经功能缺失评分

如图 1(a)所示, 与假手术组相比, 模型组 mNSS 得分更高($P<0.001$), 与模型组相比, 运动组 mNSS 得分较低($P<0.001$)。

2. Foot-Fault 评分

如图 1(b)所示, 与假手术组比较, 模型组 Foot-Fault 得分较低($P<0.001$), 与模型组比较, 运动组 Foot-Fault 得分较高($P<0.01$)。

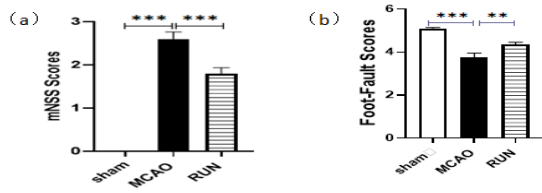


图 1 神经功能缺失评分(a)和 Foot-Fault 评分(b)(*注 : p<0.05, ** : p<0.01, ***:p<0.001)

3. TTC 染色

如图 2 (a)、(b)所示,假手术组梗死体积为 0,与假手术组比较,模型组梗死体积明显较高 (P<0.001),与模型组比较,运动组脑梗死体积稍微减少,但差异无统计学意义。

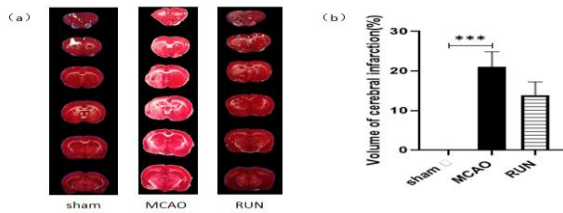


图 2 TTC 染色(a)和脑梗死体积统计(b)(*注 : p<0.05, ** : p<0.01, ***:p<0.001)

4. western blot

如图 3(a)、(b)所示,与假手术组相比,模型组 DCX 蛋白表达量降低,但差异不显著 (P>0.05),与模型组相比,运动组 DCX 表达量升高 (P<0.05);如图 4(a)、(b)所示,与假手术组相比,模型组 wnt3a 蛋白表达降低 (P<0.01),与模型组相比,运动组 wnt3a 蛋白表达增加 (P<0.01);图 5 (a)、(b)所示,与假手术组相比,模型组 β -catenin 蛋白表达量减少 (P<0.05),与模型组比较,运动组 β -catenin 表达较为升高,但差异无统计学意义。

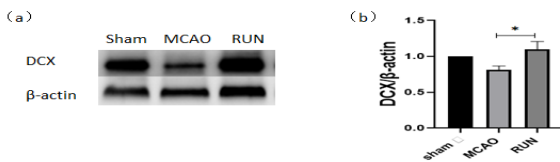


图 3 DCX 及 β -actin 显影图(a)和 DCX 蛋白表达量(b)(*注 : p<0.05, ** : p<0.01, ***:p<0.001)



图 4 Wnt3a 及 β -actin 显影图(a)和 wnt3a 蛋白表达量 (b) (*注 : p<0.05, ** : p<0.01, ***:p<0.001)

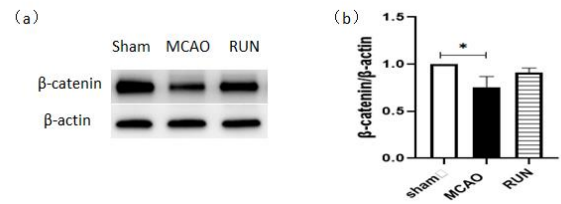


图 5 β -catenin 及 β -actin 显影图(a)和 β -catenin 蛋白表达量(b) (*注 : p<0.05, ** : p<0.01, ***:p<0.001)

Conclusion

在本研究中我们发现跑台运动不仅能改善脑缺血运动协调能力和神经功能缺损,而且在分子层面能促进 Wnt3a、 β -catenin 蛋白表达和迁移中的神经元 DCX 的表达,表明运动疗法能促进脑缺血大鼠神经功能恢复和神经干细胞动员,并且可能是通过激活 Wnt/ β -catenin 信号通路实现的。

Wnt/ β -catenin 信号通路中有众多分支和蛋白, Wnt3a、 β -catenin 是其中较为典型的,但仅有两个蛋白很难说明其是通过这条信号通路起作用的,我们还应该多加几个蛋白及抑制剂进行验证。另外,可以用免疫荧光或免疫组化进行 Nestin/BrdU 及 NeuN/BrdU 双染可以更好地说明脑卒中后神经干细胞的增殖、迁移和分化。这样才能使得实验设计更加严谨,实验结果更具有说服力。

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Comprehensive therapy based on pediatric Tuina in the treatment of Idiopathic Thrombocytopenic Purpura: A case report

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ABSTRACT

Introduction: Idiopathic thrombocytopenic purpura (ITP) is characterized by extensive skin, mucosal or visceral bleeding, and the platelet count is less than $100 \times 10^9/L$. The etiology of ITP is unclear, the pathogenesis is complex and diverse, and there is no specific drug. Tuina and moxibustion are traditional external treatments of traditional Chinese medicine, which have the advantages of safety, effectiveness, and no adverse reactions. This paper reports a clinical case of ITP treated with TCM comprehensive therapy based on pediatric Tuina with satisfactory curative effect.

Objective: To observe the clinical efficacy of pediatric Tuina in the treatment of patients with ITP and to explore the feasibility of Tuina in the treatment of ITP.

Case description: Sex, 8 years old, seeing a doctor on November 25, 2021. Two months ago, the patient suffered from subcutaneous hemorrhage in both lower limbs due to unknown causes, and the platelet count was as low as $30 \times 10^9/L$. he was diagnosed with ITP in another hospital, then received oral prednisone acetate treatment, 5mg every time, 3 times a day, after meals, treated with traditional Chinese medicine at the same time. After 2 months of treatment, subcutaneous bleeding disappeared, and platelet count increased, accompanied by obesity, dizziness, joint pain, facial acne, and other adverse reactions. With Tuina treatment, once a day, 20 minutes per time, moxibustion once a day, 20 minutes per time, and taking Prednisone Acetate, the platelet count, body weight, and adverse reactions were gradually decreased. The treatment lasted for 12 weeks and was followed up for 1 month.

Results: Subcutaneous hemorrhage disappeared, dizziness and joint pain disappeared, weight loss, facial acne cured, bright and shiny skin, good mental state, platelet count fluctuated at about $80 \times 10^9/L$, liver and kidney function examination were normal. The patients were followed up for 1 month without discomfort and could return to normal life and study.

Conclusion: The report of this case shows that under the guidance of the basic theory of traditional Chinese medicine, the comprehensive therapy of pediatric Tuina combined with moxibustion can improve the clinical symptoms and quality of life of patients with ITP.

Keywords: Chinese medicine; pediatric Tuina; Idiopathic thrombocytopenic purpura; comprehensive therapy

Introduction

Idiopathic thrombocytopenic purpura (ITP) accounts for about 30% of hemorrhagic diseases [1], which is characterized by extensive skin, mucous membrane, or visceral bleeding, and the platelet count is less than $100 \times 10^9/L$ [2,3]. The annual incidence rate of children under 18 years old is about 8.8 per 100000 people [4,5]. Under the guidance of the theory of traditional Chinese medicine, pediatric Tuina is a kind of external therapy

of traditional Chinese medicine, which is mainly used to prevent and cure diseases, strengthen the body and promote intelligence at specific acupoints or parts of children's body. Compared with drug therapy and other invasive therapy, it is safe, effective, without adverse reactions and readily accepted by children, so it is widely used in pediatric clinics [6-8].

This paper reports a case of ITP treated mainly by pediatric Tuina combined with moxibustion. After 12

weeks of treatment, the symptoms of subcutaneous hemorrhage disappeared, weight loss, dizziness, joint pain and other adverse reactions improved, platelet count increased, and the curative effect was satisfactory. As far as we know, this is the first literature report on the treatment of ITP with traditional Chinese medicine (TCM) based on pediatric Tuina.

Case report

Male patient, 8 years old, height 150cm, weight 50kg, November 25, 2021. Two months ago, there was a subcutaneous hemorrhage in both lower extremities. During the routine blood examination, the patient's platelet count was $30 \times 10^9/L$, which was significantly lower than the normal level. Other related examinations were perfected and other diseases that might lead to thrombocytopenia were excluded. Idiopathic thrombocytopenic purpura (ITP) was diagnosed in other hospitals. After confirming the diagnosis, the patients were given oral prednisone acetate treatment (Zhejiang Xianju Pharmaceutical Co., Ltd., production batch number: 190428). At the same time, combined with the internal administration of traditional Chinese medicine, after 2 months of treatment, the platelet count began to rise gradually, accompanied by weight gain, dizziness, joint pain and other adverse reactions. Out of consideration for the long-term safety of hormone drugs, the patients' families hope to seek pediatric Tuina treatment.

Before the Tuina treatment, the patient's complexion was yellow, with no luster and general mental state. The stool was thin, with the symptoms of dizziness and pain in both lower limbs complained, the platelet count was $72 \times 10^9/L$, and the liver and kidney function was normal. Physical examination showed that the patient's face showed flushing due to prednisone acetate and acne on local facial skin as figure 1. The patient denied a history of familial genetic diseases, surgical history and other diseases. In accordance with the principle of syndrome differentiation and treatment of traditional Chinese medicine, and according to the comprehensive judgment of patients' symptoms and signs and other information, we have formulated the treatment principles of warming “yang” and dredging the *Governor Meridian*, invigorating the spleen and resolving dampness.



Figure 1 The patient showed pathological obesity and redness due to taking prednisone acetate.

Intervention

Tuina therapy is performed by a tuina therapist who has been in clinical practice for more than 7 years once a day for 12 weeks. The specific operation procedures are as follows:

1. Overall conditioning

The patient took a prone position and rubbed the whole spine in the direction of Governor Meridian with Tuina method, 3-5 times from bottom to top; kneading the first sideline and second sideline of the back of *Foot-Taiyang* Bladder Meridian, 3-5 times from top to bottom; chiropractic, 3-5 times from bottom to top; kneading *Tianshu* (ST25), *Zhongwan* (CV12), *Dazhui* (GV14) and *Pishu* (BL20). with heat as the degree. 5minutes.

2. Selective stimulation of sensitized acupoints on the back

In the prone position, according to the principle of spinal neuroanatomy, the cord or nodular reaction points were found by two-handed finger-abdomen symmetrical palpation or single-hand three-finger sliding palpation, and stimulated by kneading method to the degree that the patient could bear it. Five minutes.

3. Symptomatic treatment

For lower limb joint pain, kneading Hongchi (Large Pool Point), Baichong (EX-LE3), Xuanzhong(GB39), aiming at dizziness, Pinching Fengchi (GB20), kneading Dazhui (GV14), aiming at defecation is not formed, tonifying Spleen channel line, rubbing abdomen, kneading Guanyuan (CV4), Zusanli (ST36), Weishu (BL21), each acupoint for half a minute, a total of 5 minutes.

Moxibustion was treated once a day for 20 minutes. Before treatment, we put the ignited moxa sticks (Nanyang Xiancao Pharmaceutical Co., Ltd., production batch number: XC21C06041) in the moxibustion box, and then put the moxibustion box near the Guanyuan (CV4) in the lower abdomen. After moxibustion, patients are told not to scratch or take a shower in order to avoid skin damage.

The dosage of prednisone acetate was gradually reduced according to platelet count, body weight and

adverse reactions. The dosage of prednisone acetate for the first treatment on November 25, 2021 was 5mg, 3 times a day, after meals. On December 14, 2021, the platelet count of the patient was $62 \times 10^9 / L$, the mental state was good, and the dosage was adjusted to 5mg every time, twice a day. On January 30, 2022, the platelet count was $77 \times 10^9 / L$, weight loss, and the dosage of prednisone acetate was adjusted to take 1.25mg once every other day. On February 11, 2022, the patient had a platelet count of $56 \times 10^9 / L$, continued weight loss, dizziness and joint pain relieved, facial acne improved, and stopped using prednisone acetate as figure 2. After that, he only received pediatric tuina combined with moxibustion until February 26, 2022, and were followed up for 1 month.

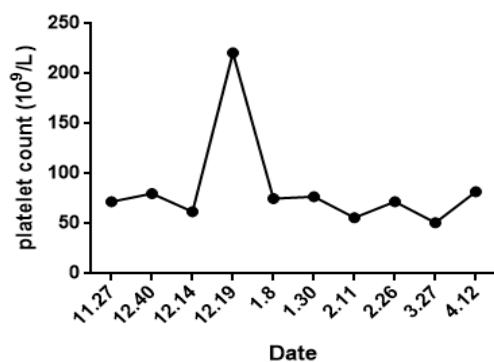


Figure 2 The changes of platelet count within 12 weeks after receiving comprehensive therapy.

Results

At the end of 12 weeks of treatment, on February 26, 2022, the patient's platelet count was $72 \times 10^9 / L$, his body weight decreased from 50kg to 45kg, liver and renal function was normal, dizziness and pain in both lower limbs disappeared, subcutaneous hemorrhage disappeared, mental health was good, facial acne disappeared, the complexion changed from dark yellow to bright and shiny as figure 3, oral prednisone acetate was stopped. During the follow-up on April 12, 2022, the patient's platelet count increased to $82 \times 10^9 / L$. the patient felt that he slept well, his spirit improved, his diet was normal, and there were no other discomfort symptoms. He felt that he had recovered to his daily life and study. We obtained the consent of the patient and signed a letter of information to release this case report and accompanying images. In accordance with the ethical standards of the Ethics Committee of the second affiliated Hospital of Yunnan University of traditional Chinese Medicine.



Figure 3 The patient's face was getting shiny and bright after 12weeks treatment.

Discussion

Traditional Chinese medicine believes that bleeding is related to the spleen. We stimulate Tianshu (ST25), Zhongwan (CV12), Dazhui (GV14) and Pishu (BL20) by pressing and kneading. These acupoints are considered to be closely related to the function of the spleen and help to restore the function of the spleen[9]. We use chiropractic techniques to stimulate the spinal nerve segments corresponding to the spleen (T11, L2) [10]. Traditional Chinese medicine believes that the spine is equivalent to Governor Meridian, which maintains the life activities of the human body, so stimulating Governor Meridian plays a positive role in promoting ITP [9]. Aiming at the symptoms of dizziness, lower limb joint pain and loose stool, acupoints such as Fengchi (GB20), Xuanzhong (GB39) and Zusanli (ST36) were selected for treatment. Guanyuan (CV4) is considered to be an important part of human body to store energy. We use moxibustion therapy to stimulate the energy of human body and promote the recovery of ITP by stimulating Guanyuan (CV4).

This case report shows that the comprehensive therapy based on pediatric tuina has a significant clinical effect on patients with ITP and may be a helpful supplementary and alternative therapy.

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Effects of Electroacupuncture on FUNDC1-LC3 Signaling Pathway in Rats Cerebral Ischemia-reperfusion Injury

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ABSTRACT

Objective: To observe the effect of electrotherapy on the FundC1-LC3 signaling pathway in cerebral ischemia reperfusion injury (CIRI) rats and to explore the mechanism of its intervention on CIRI from the perspective of mitochondrial autophagy.

Methods: 24 SD rats were randomly divided into sham operation group, model group and electroacupuncture group. In the electroacupuncture group, electroacupuncture treatment was started 24h after modeling for 30min and lasted for 3 days. The neurological function of the rats was scored before sampling. TTC staining was used to calculate the volume of cerebral ischemia infarction in rats. Western blot was used to detect LC3B and FUNDC1 protein expression in cortical region.

Results: Compared with the sham operation group, the neurological function score of the model group was significantly higher, and the neurological function deficit was serious. LC3B protein expression was increased in cortex, and the differences were statistically significant. Compared with the model group, the neurological function score of the electroacupuncture group was significantly lower, and the neurological function defect was improved. LC3B and FUNDC1 protein expressions were increased in cortical area, and the differences were statistically significant.

Conclusion: Electroacupuncture may promote the FUNDC1-LC3 signaling pathway to up-regulate mitochondrial autophagy and alleviate neurological impairment in rats, thus playing a neuroprotective role.

Keywords: electric acupuncture; cerebral ischemia-reperfusion injury; FUNDC1 - LC3 pathways; autophagy; mitochondrial autophagy

Introduction

脑中风是一种由于脑梗塞或脑出血引起的突发性脑血液循环障碍，是以脑组织损伤及相应的神经功能缺陷为主要临床表现的急性脑血管病。中风可分为两类：缺血性中风（占人口的 87%）和出血性中风（占人口的 23%），缺血性中风是最为常见的中风类型，有较高的死亡率和致残率 [1]。根据最新的全国流行病学数据，中国面临着世界上最大的中风挑战。我国脑血管病死亡率为 149.49/10 万人，2018 年死亡人数为 157 万人，位列恶性肿瘤和心脏病之后的第三大死因 [2]。

线粒体作为真核细胞中一种重要的细胞器，对维持代谢平衡、钙稳态起着重要作用；且有向组织细胞提供三磷酸腺苷（Adenosine triphosphate, ATP）、调节病理过程中的氧化代谢

以及中风后凋亡细胞死亡的作用 [3]。然而，在再灌注阶段，线粒体 Ca²⁺ 超载、线粒体通透性转换孔打开和活性氧（reactive oxygen species, ROS）过度产生，氧气和营养物质的再供应会引起线粒体损伤 [4]。失调或受损的线粒体会产生大量的 ROS 介导氧化应激并导致神经细胞死亡 [5]。

缺血缺氧状态下，线粒体蛋白 FUN14 结构域蛋白 1（FUN14 domain-containing protein1, FUNDC1）通过介导介导线粒体自噬，选择性清除受损线粒体，从而起到神经保护作用 [6]。因此，本研究通过建立 CIRI 大鼠模型，以受体 FUNDC1 介导的线粒体自噬微切入点，从线粒体自噬角度探讨电针干预 CIRI 的作用机制。

Methodology

1. 材料和方法：

1.1 动物

SPF级雄性SD大鼠24只，250-280g，环境温度22-25℃，分笼、标准饲料饲养。

1.2 主要试剂

RIPA裂解液，货号为P0013B，上海碧云天生物技术有限公司；苯甲基磺酰氟（PMSF），货号为P0100北京索莱宝科技有限公司；BCA蛋白定量试剂盒，货号BL521A，白鲨致力科研有限公司；LC3B、FUNDC1、山羊抗兔IGg，货号分别为ab192890、ab224722、ab6721，英国Abcam公司。

1.3 造模、分组、治疗

24只大鼠随机分为假手术组（Sham）、模型组（MCAO）、电针组（EA），每组8只。采用线栓法[7]制备大鼠左侧大脑中动脉缺血再灌注模型（MCAO），实验步骤简述如下：大鼠进行异氟烷麻醉后（瑞沃德小动物麻醉机），正中切开颈部皮肤，钝性分离颈部肌肉暴露颈总、颈外和颈内动脉，于颈外动脉远端结扎，颈外动脉切开一小口插入栓头包被硅胶4-0尼龙线栓，线栓经颈内动脉进入颅底达到大脑中动脉处造成相应脑区缺血，60分钟后拔出线栓实施再灌。造模过程中控制直肠温度处于37±0.5℃。假手术组动物除不插入和拔出线栓，其余各步骤均相同。术后24小时通过神经功能缺失评分，大于2分的大鼠纳入实验（假手术组除外）。EA组每天14:00-15:00麻醉后选取百会、大椎、曲池、内关进行电针治疗30min（电流2mA，1寸华佗牌针灸针），假手术组和模型组做同样时长的麻醉，但不进行电针治疗。

1.4 神经功能评分

取材前采用7分制[8]测试动物整体神经功能缺失状况，用于评估神经功能恢复情况。具体评分标准为：轻提大鼠尾巴，观察前肢弯曲状况，双前肢伸向地面且无其它神经病学特征者评为0分；提大鼠尾时其对侧前肢持续弯曲而无其他异常者评为1分；将鼠置于软塑封纸上，在其肩膀后轻轻侧推直至前肢侧滑数英寸，重复数次，抵抗推力的能力下降者被评为2分；在地面上活动时略向上拉其尾，如向瘫痪侧旋转者评为3分；自行行走时即向瘫痪侧旋转者评为4分；不能自行行走者评为5分；无自发活动评为6分；死亡评为7分。

1.5 TTC染色

再灌注1天便开始电针治疗，持续3天后，用10%水合氯醛（0.35ml/100g）腹腔注射麻醉大鼠，断头取脑并置于大脑切片模具中，用手术刀片进行2mm连续切片，置于0.1%TTC中，恒温箱中37℃孵育15-30min。

1.6 Western blot 检测

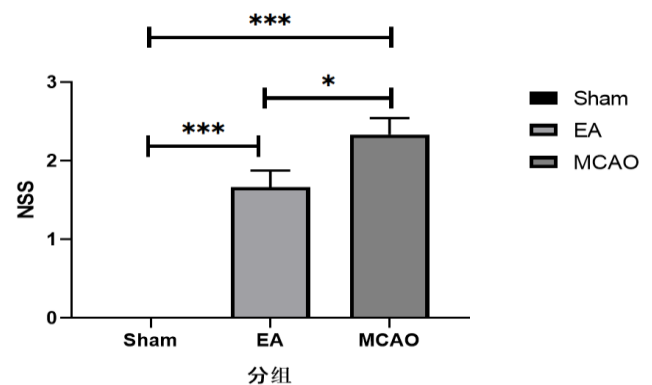
取皮层区域脑组织置于冰上，加入裂解液（RIPA裂解液：PMSF=99:1），高速低温组织研

磨仪充分研磨，提取总蛋白，BCA测定蛋白浓度，各组取10ug蛋白上样、经电泳、转膜、奶粉封闭，加LC3B（1:3000）或FUNDC1（1:2000）4℃一抗孵育过夜、二抗孵育1h后经化学发光成像系统分析并计算蛋白灰度值，用目的蛋白与内参的灰度值来表示蛋白相对表达量。

Results

1. 神经功能评分

假手术组无神经功能缺损，与假手术组比较，模型组大鼠神经功能评分明显升高，神经功能缺损严重，差异有统计学意义（ $P<0.001$ ）；与模型组比较，电针组大鼠神经功能缺损降低，差异有统计学意义（ $P<0.05$ ）。见图一

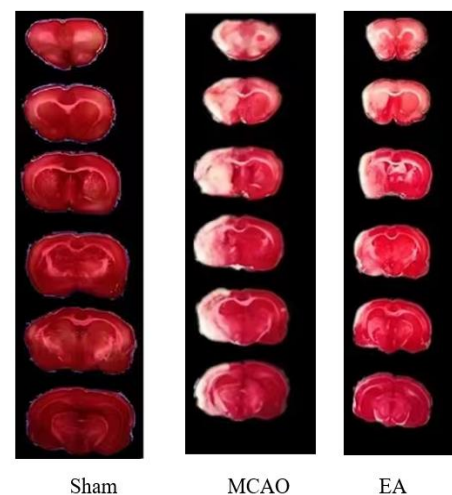


图一：神经功能评分

注：*** $P<0.001$ ；** $P<0.01$ ；* $P<0.05$

2. TTC染色

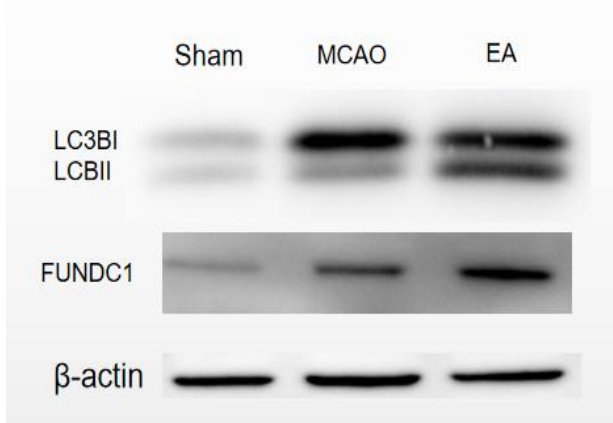
见图二



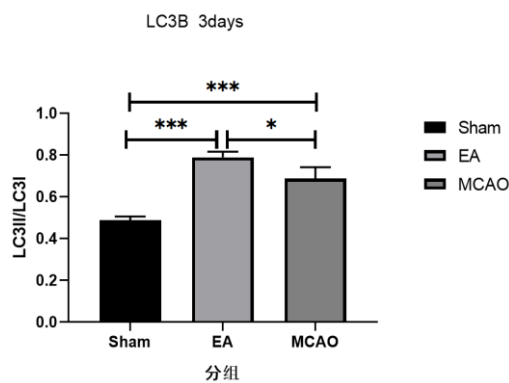
图二：TTC染色

3. 电针对 CIRI 大鼠皮质区 LC3B、FUNDCl 蛋白表达的影响

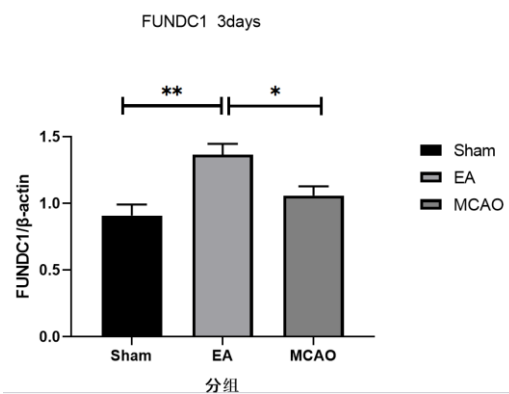
Western blot 结果显示, 与假手术组比较, 模型组大鼠皮质区 LC3B 表达升高, 差异有统计学意义 ($P < 0.001$) ; 与模型组比较, 电针组大鼠皮质区 LC3B、FUNDCl 蛋白表达升高, 差异有统计学意义 ($P < 0.05$) 。见图三、四、五。



图三



图四



图五

Discussion

脑缺血再灌注损伤后, 线粒体三磷酸腺苷 (adenosine triphosphate, ATP) 生成障碍、Ca²⁺超载, 进一步诱导谷氨酸兴奋毒性, 引发一系列级联反应从而诱导细胞凋亡发生[9]。在脑血流再通后, 复氧促使线粒体产生大量活性氧 (reactive oxygen species, ROS), 从而导致氧化应激损伤、诱导细胞凋亡[10]。此外, ROS 过度释放及 Ca²⁺超载等因素可促进促炎因子的释放, 从而导致脑组织炎症反应[11]。脑中风后线粒体自噬可以清除受损或功能障碍的线粒体来抑制氧化和凋亡级联反应, 激活适度的线粒体自噬可能是脑中风后的治疗重要靶点。

研究表明电针在自噬的整个过程中起着关键作用, 其中包括自噬的启动、囊泡成核、自噬体的扩张和成熟以及自噬溶酶体的融合和降解[12]。如: Wang 等利用大鼠 MCAO 模型也证实了电针可以调控线粒体自噬水平进而保护神经细胞免受 ROS 氧化应激损伤[13]。以上表明电针具有调控线粒体自噬保护神经细胞的作用, 但其作用机制尚不完全清楚。因此, 阐明电针调控线粒体自噬的作用机制对脑中风的的基础研究和临床治疗具有重要意义。LC3 是自噬标志蛋白, 包括 LC3I 和 LC3II, 两者可以相互转化。FUNDCl 是位于线粒体膜上的受体蛋白, 缺氧情况下可介导线粒体自噬。该研究表明, 电针可上调 LC3B、FUNDCl 蛋白表达水平, 激活线粒体自噬。

Conclusion

综上, 电针可能通过促进受体 FUNDCl 介导的线粒体自噬通路, 改善 CIRI 神经功能症状, 为阐明电针减轻 CIRI 损伤的机制提供实验依据。

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Research Progress on Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells induced by Traditional Chinese Medicine Monomer

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ABSTRACT

As the population ages, osteoporosis has become a global bone disease, increasing the risk of fractures and seriously affecting human health. Bone marrow mesenchymal stem cells (BMSCs) have the potential to differentiate in different directions. Inducing osteogenesis and inhibiting adipogenic differentiation are key to preventing and treating osteoporosis. Traditional Chinese medicine (TCM) is a bone metabolic disease with obvious therapeutic effects. Research results show that the part between traditional Chinese medicine and its purification material can effectively induce bone marrow mesenchymal stem cells to osteoblast differentiation. Focusing on the physiological characteristics of BMSCs, this paper summarized the mechanism of TCM monomers in osteogenic differentiation.

Keywords: bone marrow mesenchymal stem cells; Osteoblasts; Traditional Chinese medicine (TCM); differentiation

Introduction

骨髓间充质干细胞 (one marrow mesenchymal stem cells, BMSCs) 被称为多能细胞, 由于自身可通过不断更新、分化被广泛应用于治疗各类疾病, 具有分化为多种细胞类型的潜能, 如成骨细胞 (Osteoblast, OB)、软骨细胞、脂肪细胞、骨细胞、成肌细胞等 [1], 其中成骨细胞参与骨形成。有研究显示, 随着年龄的增长, BMSCs 更倾向于分化为脂肪细胞而不是成骨细胞并发生衰老, 导致骨形成减少从而成为骨质疏松 [2]。BMSCs在一定条件下可诱导分化成为OB, 主要负责骨基质的合成、分泌和矿化, 从而实现骨的再生。

现如今寻找一种快速简便的方法从BMSCs中获取成骨细胞是一个热门研究话题, 因此从BMSCs成骨诱导剂着手, 可能有助于寻找新的方案。目前 BMSCs成骨诱导剂多使用细胞因子、激素类、生物材料等, 通过体外培养 BMSCs或移植等外源性途径诱导其向成骨方向分化, 有助于维持骨代谢动力平衡, 但缺乏最佳培养条件以及移植数量的不确定性, 同时考虑费用

昂贵的问题, 使其应用受到一定限制。长久以来中药治疗骨代谢疾病疗效显著, 有研究表明部分中药及其有效成分对于BMSCs向OB增殖和分化具有促进作用, 且在一定条件下, 促BMSCs分化为OB方面优于雌激素 [3]。目前中药诱导成骨疗效好且安全性高, 但其具体机制仍不清晰, 同时中药成分众多, 使对中药单体诱导 BMSCs 向成骨分化的研究较少, 特对此相关研究作一总结。

Literature review

中药单体诱导BMSCs成骨分化

1. 淫羊藿苷

淫羊藿苷(Icariin, ICA)是中药淫羊藿的主要活性成分之一, 具有补肾阳、强筋骨、抗衰老等功效。ICA 具有多种药理活性, 包括类激素、抗肿瘤、免疫调节和抗氧化作用 [4]。研究表明淫羊藿苷能够有效防止骨丢失、增加骨再生, 从而具有调节骨代谢的作用 [5]。Xue, L等 [6]通过相关研究指出淫羊藿苷是用于治

疗骨质疏松症的多靶标化合物，涉及启动成骨细胞生成并抑制脂肪生成和防止破骨细胞分化。

淫羊藿苷发挥活性作用的途径之一就是诱导 BMSCs 成骨分化，Yang A 等 [4] 认为 ICA 在 BMSCs 中的主要作用是促进增殖和成骨，并通过多种信号途径介导，包括 MAPK/ERK/p38、Wnt/ β -catenin、PI3K/Akt/eNOS/NO/cGMP/PKG、MAPK 等途径作用于成骨细胞，此外，ICA 通过 PI3K、ERK1/2 和 EGF-EGFR 途径促进血管生成，并通过 MAPK 途径促进 BMSCs 迁移。研究者指出 ICA 通过 PI3K/Akt/eNOS 信号传导途径，不仅在体外激活内皮血管生成，而且在体内直接刺激血管生成 [7]，从而促进 BMSCs 向 OB 分化、抑制其成脂分化，以促进 OB 的成骨活性。Zhang, S [8] 等发现 ICA 可以抑制成骨细胞-破骨细胞共培养物中 BMSCs 的成脂分化，并促进成骨细胞分化并抑制破骨细胞分化。

2. 骨碎补总黄酮

骨碎补(Rhizoma Drynariae)为骨科常用中药，最早记载于《本草拾遗》，其性温、味苦，归肾、肝经，有补肾强骨、散瘀止痛等功效，常用于治疗肾虚腰痛、骨折筋伤、牙齿松动等。研究显示，骨碎补能够有效促进骨形成，在防治骨代谢疾病方面效果显著，其有效活性成分骨碎补总黄酮(Total flavonoids of Rhizoma Drynariae, TFRD)已被证明可有效治疗骨质疏松、骨折、骨缺损以及骨关节炎 [9]。TFRD 能够通过多条信号途径促进 BMSCs 成骨分化、OB 成熟并抑制破骨细胞活性来有效地促进骨形成，现已将此开发上市，成为中药二类新药(强骨胶囊)，临床疗效也得到广泛肯定 [10]，相关证据证实 TFRD 在改善骨质量方面有一定作用 [11]。史岩等 [12] 通过实验得出结论：TFRD 对骨质疏松性骨折的治疗作用，很可能是通过活化 Wnt/ β -catenin 信号通路，从而促进 BMSCs 向成骨分化而实现的。孙奇峰等 [13] 在模拟失重状态下，发现 TFRD 能够激活 MAPK/ERK 通路使 BMSCs 向 OB 增殖、分化成熟。TFRD 也可通过阻断 BMPR-1A 信号传导和增强 BMPR-1B 信号传导来抑制 BMP-2 和 BMPR-1A 的结合，从而控制细胞分化 [14]。

骨碎补提取物中，柚皮苷(Naringin)是一种明确具有应用潜力的物质结构，柚皮苷可增加骨形态发生蛋白(BMP)的体外表达以及 Wnt/ β -catenin 和细胞外信号相关激酶(Erk)通路的激活，从而促进了 OB 的增殖和分化，同时还具有通过修饰 RANK/RANKL 相互作用并在体外诱导破骨细胞凋亡来抑制破骨细胞生成的作用 [15]。Lin, F X 等 [16] 研究表明，柚皮苷通过 IHH 信号通路上调 Foxc2，促进 BMSCs 向成骨分化。也有相关研究显示 50mg/mL 的柚皮苷可以增加成骨基因的 mRNA 水平和 Notch1 表达，同时降低 PPAR γ 2 mRNA 的水平，表明柚皮苷的成骨作用部分涉及 Notch 信号传导途径影响 BMSCs 成骨分化 [17]。

3. 大黄素

大黄素(Emodin, ED)是大黄、何首乌和虎杖的主要有效成分，具有抗菌、抗肿瘤、抗炎、保护器官等多种药理作用 [18]，除此之外 ED 还具有骨重塑的作用 [19]，一部分作用来自诱导 BMSCs 分化，且分化

后的细胞具有 OB 特点。有研究直接显示 ED 可以抑制 BMSCs 成脂分化，促进成骨分化，增加去卵巢小鼠的 OB 数量，骨矿物质密度(BMD)、骨小梁体积分数(BV/TV)、小梁数(Tb.N)和连接密度(Conn.D)，减少骨髓脂肪组织和脂肪细胞，诸如 Runx2、Osterix、I 型胶原、骨钙蛋白(OCN)或碱性磷酸酶(ALP)等成骨标记的基因和蛋白质的表达上调，同时，参与脂肪形成的基因和蛋白质，PPAR γ 、C/EBP α 和 β 2 被下调 [20]。有研究者分析其原因，认为 ED 诱导成骨分化可能是通过调节细胞周期的分布，细胞凋亡和血小板生成素的表达来促进 BMSCs 的增殖 [21]。Lee, SU 等 [22] 发现低浓度 ED 可以通过激活 PI3K/Akt 信号通路，诱导 BMP-2 mRNA 表达、ALP 的表达和矿化，ED 也可通过激活 BMP-Smad 信号轴，诱导 BMP-9 mRNA 表达，从而增强 OB 的分化 [18]。从何首乌中提取的 Emodin-8-O- β -D-glucoside 也被证实可刺激 MC3T3-E1 的增殖和分化 [23]。

4. 补骨脂素和异补骨脂素

中药补骨脂具有温肾助阳、纳气平喘、温脾止泻的作用，补骨脂素(Psoralein, PRN)和异补骨脂素(Isopsoralein, IPRN)为补骨脂中的主要化学活性成分，两者具有多种生物学作用，包括抗骨质疏松作用。PRN 通过刺激 BMSCs 向成骨分化，在卵巢切除术引起的骨质疏松大鼠中具有抗骨质疏松作用 [24]，有研究证实其作用来自 PRN 激活 BMP、MAPK 和 IRE1 信号途径以此促进 OB 的增殖和分化 [25]。IPRN 作为 PRN 的异构体，可通过充当 AhR 拮抗剂并通过 AhR/ER α 轴促进 OB 分化，以此增加骨强度和骨小梁结构 [26]。同时 IPRN 也可从多种水果和蔬菜中分离得到，通过对骨形成的刺激作用，促进 BMSCs 的成骨分化 [27]。Wang, J 等 [28] 在 IPRN 处理卵巢切除小鼠后，观察到 ALP、OCN 和 Runx2 的表达水平上调，而 PPAR γ 和 C/EBP β 的表达下调，同时在体外和体内，mTORC1 信号转导也受到抑制，可以认为 IPRN 促进 BMSCs 的成骨分化而抑制成脂分化。相关研究也表明 IPRN 浓度为 1*10⁻⁵mol/xL-1 可以促进 BMSCs 向 OB 的分化 [29]。

5. 黄精多糖

黄精多糖(Polygonatum sibiricum polysaccharide, PSP)是中药黄精的主要活性成分之一，目前已证实黄精具有抗氧化、抗骨质疏松、抗肿瘤等治疗功效，在其抗骨质疏松的机制中，通过多种信号途径达到促 BMSCs 成骨分化、促 OB 矿化以及抑制破骨细胞生成的作用 [30]，已被广泛研究。Du, L 等 [31] 通过实验认为 PSP 可以通过增加 β -catenin 的积累来促进小鼠 BMSCs 中的 OB 分化并阻断破骨细胞的生成，且认为 PSP 是通过 ERK/GSK-3 β / β -catenin 信号通路在体外促进 OB 分化和矿化 [32]。Zong, S 等 [33] 实验结果表明，PSP 可显著升高 ALP、骨钙素(Osteocalcin, OC)、I 型胶原蛋白(PINP)和 BMP-2 的表达水平，认为 PSP 可促进成骨分化过程中 BMSC 的增殖并增强其生存能力，以此达到治疗骨质疏松的作用。

6. 蛇床子素

中药蛇床子，有燥湿祛风、杀虫止痒、温肾壮阳之功效，其主要活性物质蛇床子(Osthol,Os)在以往研究中已被证实具有抗炎镇痛、骨保护、促进骨形成的作用 [34]，在防治骨质疏松方面具有一定成效。相关文献报道Ost防治骨质疏松的机制是通过介导骨保护素/受体激活剂核因子 κ B 配体(OPG/RANKL)、Wnt/ β -连环蛋白、骨形态发生蛋白(BMP)及Notch信号通路相关 [35]。体外研究表明，Ost可激活Wnt/ β -catenin信号传导，增加BMP-2的表达并刺激OB分化，研究者还发现 β -catenin和Bmp2基因的靶向删除消除了分化过程中的刺激作用，建议Ost通过 β -catenin-BMP信号传导来促进成骨分化 [36]。有一研究通过观察Ost对小鼠成骨MC3T3-E1细胞和小鼠股骨骨折修复的骨促进作用，认为Ost介导的成骨作用与cAMP/CREB信号通路的激活和下游因子osterix的表达有关，无论在体外还是体内，Ost显示出明显的促成骨作用 [37]。Zheng, X等 [38]实验结果表明Ost可以上调自噬相关基因Beclin1和LC3的蛋白质和mRNA表达水平以此增加BMSCs自噬，从而促OB分化。

7. 白藜芦醇

白藜芦醇(Resveratrol, Res)广泛存在于中药虎杖、花生、葡萄以及浆果等自然植物中，具有抗氧化、抗肿瘤、调节血脂等作用，同时能与雌激素受体结合而发挥作用 [39]，被视为植物雌激素，对绝经后骨质疏松症的治疗有一定功效。在最近的一项研究中，数据表明低剂量Res的补充可显著改善绝经后妇女的腰椎和股骨颈骨密度，并降低骨吸收标记物CTX的表达 [40]。近年来也有相关研究表明Res在BMSCs的细胞活力、增殖、凋亡、多能性和成骨成脂分化中起着至关重要的作用 [41]，Res不仅参与了体外MSC的成骨、脂肪形成和神经调节，且会增加NO的产生和cGMP的含量并激活ER介导的细胞外信号，最终导致MSC的生长和成骨分化呈剂量依赖性增加 [42]。但是Res对MSC的培养具有矛盾作用，根据其细胞传代和SIRT1的表达对其呈现相反的作用，通过对 β -catenin的调节可增强早期传代的MSC自我更新潜能和多能性，也可加速晚期传代的MSC细胞衰老，故Res可有效应用于早期传代的MSC培养，并在此过程中应考虑到细胞传代和SIRT1表达等因素 [43]。

8. 其他

其他天然植物中有部分也具有促BMSCs的成骨作用，如从多味补肾中药中均能提取到的槲皮素，已被证实能够在骨分化中起重要作用。研究表明槲皮素糖苷Q3G分别通过Wnt/BMP和PPAR γ 途径刺激了人骨髓间充质干细胞中的OB分化并抑制了脂肪细胞分化 [44]。还有从芦荟中提取出的芦荟素也发挥着同样的作用，在成骨诱导条件下芦荟素会增加ALP活性，促进BMSCs矿化，同时增加成骨相关基因的表达，最终结果表明芦荟素通过激活ERK1/2-Runx2信号通路来促进BMSCs的成骨分化 [45]。也有部分研究者将目光放在姜黄素的骨代谢调节作用上，除了抗氧化、抗炎、抗肿瘤和神经保护的特性外，有研究表明姜黄素对BMSCs具有良好的调节作用 [46]，在5mmol/L姜黄素处理后能够有效抑制人骨髓间充质干细胞的成脂分化

并增强成骨分化 [47]，目前对此研究相对较少，仍需更多的数据支持和临床验证。

Discussion and conclusion

不足

1.虽然有很多诱导因素可诱导BMSCs分化成为OB，但其分化机理并没有完全清楚，特别是对于BMSCs定向分化的调节和控制；2.对分化诱导出的OB鉴定标准仍有待人们深入研究和探索；3.目前促BMSCs向OB分化增殖的相关研究中，中医药对其干预作用的研究还远远不够，对各种中药产生作用的生物学机制和具体有效成分的分析仍相对不足，以及副作用和相关影响因素的发现需要更多的研究。

展望

以往研究从不同角度对BMSCs的成骨分化的具体机制及分化调控进行了深入的研究，取得了较大的进展，可以看出骨髓间充质干细胞在骨组织工程中有着很好的应用前景。中药在化学结构和生物活性上表现出更多的多样性，并且毒性更低。因此，中药可能作为具有应用潜力的成骨诱导剂新来源。在今后的相关研究中，中药诱导BMSCs向成骨分化时，应进行对比研究，筛选出影响最明显的药物，继而深入到单体和有效成分的角度并对其分子结构进行研究，在机制方面要对影响的基因和蛋白组学进行研究，以便明确其信号转导通路和具体作用机制。

通过上述相关研究，从现代科学角度支持和解释中医药，为我国传统中医药治疗骨质疏松提供了科学依据。同时，也有望在中医药领域开发出能够促进BMSCs成骨分化的有效药物，为骨质疏松的防治提供新的治疗方法。

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Traditional Chinese Medicine in Treating Depression

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ABSTRACT

Depression is a mental health disease that causes patients to be in a negative emotional situation. It is a psychosomatic disease that is difficult to recover from and has a high recrudescence rate. Much research and clinical results showed that Traditional Chinese Medicine (TCM) is effective in depression and can reduce the symptoms. A depression patient with depression came for acupuncture treatment. After four acupuncture treatments, the emotion turned more positive, and symptoms such as insomnia, poor appetite, body pain, etc., had reduced. These cases showed that TCM is an effective and alternative option for depression.

Keywords: *Traditional Chinese Medicine, Herbal, Acupuncture, Depression, Case*

Introduction

Depression is a common mental illness that significantly burdens public health with a higher risk of dementia, premature mortality from physical disorders, and maternal depression impacting child growth and development [1]. Depression patients will suffer from depressed emotion (sadness, irritability, emptiness) or loss of pleasure or interest in activities for most of the days for at least two weeks. The factors of depression included life events (childbirth, bullying, life stress), diseases (thyroid diseases, menopause), personality, etc. [2-4]. Reports showed that 3.8% of the population has depression, and it had an 18% increase in depression patients between 2005 and 2015 [5-7]. In Thailand, a report showed that 4.3 million adolescents between 13-17 years old, 6.4% of the total population, had signs of suicidal behavior, anxiety, loneliness, etc. [8].

Depression in TCM

In TCM theory, depression is called "Yu Zheng." The main symptoms of "Yu Zheng" are depressed emotion, chest tightness sensation, emotional fluctuation, etc. [9]. Depression is separated into Liver Qi Stagnation, Liver Stagnation with Spleen Deficiency, Heart and Spleen Deficiency, Kidney Deficiency with Liver Stagnation, and other syndrome types according

to TCM theory [10]. The factors that cause depression in TCM are severe and sudden emotional changes that affect Qi circulation and the viscera organ function (Liver, Heart, Spleen, and Kidney).

TCM herbal treatment

Articles from 2007 until 2019, the standard formulas used in the treatment of depression are Wendan Decoction, Banxia Houpu Decoction, Xiaoyao Powder, etc. Moreover, Fuling (*Poria*), Gancao (*Radix Glycyrrhizae*), Chaihu (*Radix Bupleuri*), Banxia (*Rhizoma Pinelliae*), etc. are common herbs used in the treatment of emotional diseases [11].

In treating depression, Wen Dan Decoction and Xiaoyao Powder could regulate monoamine neurotransmitter levels, anti-inflammation reaction, hypothalamic-pituitary-adrenal axis, etc. [12,13]. Randomized Clinical Trial (RCT) research showed intervention group had better results than the control group [14,15].

TCM acupuncture treatment

A study showed that between 2010 to 2020, 234 research studies about TCM treatment on depression [16]. The study also showed that 54.70% of 234 research on acupuncture treating depression, TCM combination

treatment was 23.93%, and a combination of TCM with western medicine treatment was 20.09%. Acupuncture showed good effects on depression [17]. The most common acupoints were Bai Hui (DU 20), Yin Tang (EX-HN 3), Nei Guan (PC 6), San Yin Jiao (SP 6), Sishenchong (EX-HN 1), and Shenmen (HT 7) [18].

Research showed that Baihui (DU 20) and Shentang (BL 44) were the common acupoints in the early stage of depression. In mid-stage, Neiguan (PC 6), Shuigou (DU 26), Baihui (DU 20), Yintang (EX-HN 3), etc. will be the common acupoints. In the late stage, Si Shen Chong (EX-HN 1), and Shenting (DU 24) will be the common acupoints for the treatment [19].

The acupoints called "Thirteen Ghost Points" (TGP) have been used for emotional diseases since ancient China. There are Renzhong (DU 26), Shaoshang (LU 11), Yinbai (SP 1), Daling (PC 7), Shenmai (BL 62), Fengfu (DU 16), Jiache (ST 6), Chengjiang (RN 24), Laogong (PC 8), Shangxing (DU 23), Huiyin (RN 1), Quchi (LI 11), Jinjin (EX-HN 12), Yuye (EX-HN 13), Shenting (DU 24), Xingjian (LR 2), Yanglingquan (GB 34), Ruzhong (ST 17), and Jianshi (PC 5) [20]. They brought good effects on the treatments of neuropsychological disorders [21]. Research showed regional homogeneity and amplitude of low-frequency fluctuation value decreased after laser acupuncture on TGP. The results mean the treatment could induce alternations of spontaneous brain functions and affect neuropsychological diseases [22].

Gui Ku acupoint from *Beiji Qianjin Yaofang* (*Prescriptions Worth a Thousand Pieces of Gold for Emergencies*) is a common acupoint that is used for emotional diseases. It is located on the nail's angle on the thumb's radial side and the big toe's medial border. The specific operation of moxibustion at Gui Ku point is first to put the two thumbs together, keep the front edge of the nail in the side-to-side position, then moxibustion with wheat grains until the patient feels hot, and then remove it. It is one of the most common acupoints for emotional diseases in ancient times. It showed good results in the treatment of depression [23,24].

Some studies have shown that moxibustion at Shaoshang (LU 11) and Yinbai (SP 1) points has a good curative effect on phlegm-Qi stagnation type depression. The Hamilton Depression Rating Scale (HAMD) scores of patients who received moxibustion on LU 11 and SP 1 combined with western medicine treatment were significantly lower. The overall curative effect was 16.7% better than the control group [25].

Case Report

23 years old male depression patient with depressed emotion, sluggishness, anhedonia, insomnia, poor appetite, body pain, red tongue with tooth marks, white greasy tongue coating, and string pulse. The psychologist in other hospitals diagnosed the depression

level. The Chinese syndrome differentiation is considered Liver Stagnation with Spleen Deficiency. The patient accepted acupuncture treatment with inserted needles on Shangxing (DU 23), Sishenchong (EX-HN 1), Yin Tang (EX-HN 3), Hegu (LI 4), Tianshu (ST 25), Sanyinjiao (SP 6), and Taichong (LR 3). The needles were kept for 30 mins per treatment. After 4 treatments, the patient felt the emotion more stable and symptoms relieved. The psychologist diagnosed as the level of depression decreased.

The main idea of the treatment was to soothe the liver and regulate Qi, smooth meridians, and strengthen the spleen and stomach. EX-HN 1, EX-HN 3, and DU 23 are the acupoints for negative emotion relief and common acupoints for insomnia [26,27]. The negative emotion of the patient was from study stress and worried about the examination. In TCM theory, worry emotion will damage spleen function. The study showed that mental would affect the gastro-intestine function [28]. ST 25 can strengthen spleen function and relieve the symptoms of poor appetite [29]. LI 4 and LR 3 are the acupoints to smooth the Qi flows and can relieve depressed emotion [30].

Conclusion

TCM treatments effectively control negative emotions and have been used for years. The treatments can enhance negative emotional problems and bring good clinical application effects.

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**Full proceeding
for E-poster presentation**

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Data Analysis of Acupoint Selection in Acupuncture Treatment for Post-Stroke Depression Based on Traditional Chinese Medicine Inheritance Support System (TCMISS)

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ABSTRACT

Introduction: Post-Stroke Depression (PSD) is the one of the most common clinical complications in stroke patients. There are currently many methods of treatment for this disease, for Traditional Chinese Medicine acupuncture will be used mainly because it is safe, effective and few side effects.

Objective: To summarize the rule of acupoint selection in acupuncture treatment of post-stroke depression based on data mining of clinical literature papers by Traditional Chinese Medicine Inheritance Support System (TCMISS) to provide new insights in clinical therapy.

Methods: The clinical research literature on acupuncture treatment of post-stroke depression was collected from the CNKI, Weipu and Wangfang Journal database between May 2012 to April 2022. The acupoint prescription was entered into the “Traditional Chinese Medicine Inheritance Support System (V2.5) software” and were analyzed for frequency, repetitive and core combination of prescribed acupoints.

Results: 85 articles, 85 acupoint prescriptions and 79 acupuncture points were included into the study. The acupoints with highest frequency in overall usage in descending order are Baihui (DU20), Taichong (LR3), Neiguan (PC6), Shenmen (HT7) and Sishenchong (EX-HN1). The high-frequency acupoints combinations are listed as follows: Baihui (DU20) and Neiguan (PC6), Baihui (DU20) and Taichong (LR3), Baihui (DU20) and Shenmen (HT7).

Conclusion: Data mining and analysis of acupoint selection in acupuncture treatment of post-stroke depression could provide a new idea and insight for further clinical treatment.

Keywords: Post-stroke depression; Acupuncture; Acupoint selection; TCMISS

Introduction

Post-Stroke Depression (PSD) is a secondary depression that occurs after stroke and is the one of the most common clinical complications in stroke patients. The main features are depression, slow thinking, disturbance of thinking content and reduced conscious activities, even some patients have behaviors such as world-weariness and suicide, which affect the recovery of patients [1]. The incidence of PSD is 30% to 50% [2]. As for the treatment of this disease, western medicine mostly uses antidepressant drugs for antagonistic treatment. These drugs have achieved certain effects,

but there are also different degrees of adverse reactions, which are easy to cause allergic reactions and the formation of dependence, resulting in poor patient compliance, thereby affecting patient treatment [3]. Traditional Chinese medicine is mainly based on prescriptions and acupuncture in the treatment methods, and its clinical application has the advantages of obvious curative effect, high safety and less side effects. Relevant studies have shown that acupuncture to stimulate the cerebral cortex can effectively increase the content of NE, 5-HT and DA in the blood, and at the same time can inhibit the hypothalamus-pituitary-

adrenal cortex axis, improve and effectively inhibit nerve cell apoptosis, thereby improving PSD symptoms and restoring neurons damage [4]. In this paper, with the aid of Traditional Chinese Medicine Inheritance Support System (V2.5), the data mining method will be used to explore the selection rules of acupuncture points for post-stroke depression, provide reference and inspiration for clinical treatment based on data correlation.

Methodology

Research literature selection

Searched in CNKI, Weipu, Wangfang and selected related documents in the database between May 2012 to April 2022.

- a. Inclusion criteria : Clinical treatment of acupuncture for PSD published in domestic journals, Observation; Diagnosis of PSD; Acupuncture treatment the main treatment is effective; Use of Chinese or internationally recognized diagnostic and efficacy evaluation standards
- b. Exclusion criteria : Non-clinical research literature such as animal experiments, literature reviews, experience summaries; Issuing other diseases after stroke such as constipation and dizziness; Conference papers, Master's and Doctoral thesis ; Documents using non-traditional acupuncture, such as acupoint injection, scalp acupuncture, electronics, floating needles; case report; Repeated publications.

Retrieval strategy

Use " Post- Stroke Depression AND acupuncture" as the key word for the search

Terminology specification

Use "The Name of Acupoint" and "Positioning" "standardizes the acupuncture point names to classical names", If you find the acupoint name "Shuigou (GV26)" and "Renzhong (GV26)" record as "Renzhong (GV26)"

Statistical processing

Use the data analysis "Frequency statistics" tools in TCMISS function. The frequency of usage of different acupuncture points were analysed. Acupoint frequency was sorted from high to low. These were based on association rules that were applied to the "composition formula" for the analysis of the compatibility of each acupuncture points. See figure 1, figure 2.



Figure 1 Traditional Chinese Medicine Inheritance Support System (TCMISS)



Figure 2 Traditional Chinese Medicine Inheritance Support System (TCMISS)

Results

Frequency of acupoint selection

A total of 85 acupoints were used in 85 research literature and the total usage of acupoints were 583 times. Use of "Frequency statistics" to calculate the frequency of use of each acupoints. Excel was used to find the frequency as well and return with the 17 most used acupoints. The top 5 acupoints according to the frequency were Baihui (DU20), Taichong (LR3), Neiguan (PC6), Shenmen (HT7) and Sishenchong (EX-HN1). See table 1, figure 3

Table 1 Acupoint frequency for acupuncture treatment of PSD

No.	Acupoint	Frequency	%	Meridian
1	百会 (DU20)	55	69.62%	Governing Vessel
2	太冲 (LR3)	46	58.23%	Yin
3	内关 (PC6)	39	49.37%	Yin
4	神门 (HT7)	34	43.04%	Yin
5	四神聪 (EX-HN1)	30	37.97%	Extra point
6	神庭 (GV24)	28	35.44%	Governing Vessel
7	三阴交 (SP6)	24	30.38%	Yin
8	印堂 (EX-HN6)	20	25.32%	Extra point
9	足三里 (ST36)	19	24.05%	Yang
10	膻中 (CV17)	18	22.78%	Renmai
11	肝俞 (BL18)	17	21.52%	Yang
12	人中 (GV26)	17	21.52%	Governing Vessel
13	心俞 (BL15)	15	18.99%	Yang
14	合谷 (LI4)	14	17.22%	Yang
15	肾俞 (BL23)	14	17.22%	Yang

No.	Acupoint	Frequency	%	Meridian
16	脾俞 (BL20)	12	15.19%	Yang
17	阳陵泉 (GB34)	11	13.92%	Yang

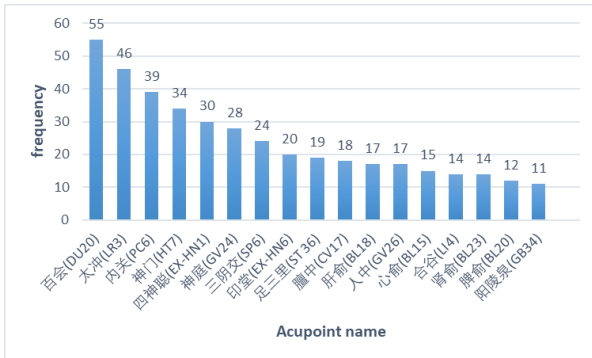


Figure 3 Acupoint frequency for acupuncture treatment of PSD

Analysis of the rule of acupoint selection

In the “Rule of acupoint group” module, the amount for analysis was set to 20 (in the samples were less than 20 entries) and confidence level at 0.6 (Indicating a confidence level of over 60%). The top 10 mostly used acupoint combinations are shown in table 2 and figure 4. The rule of compatibility came out with 10 results is shown in table 3. Results from the “Show table” function of the acupoint grouping are shown in figure 5 (Support 20%), figure 6 (Support 30%) and figure 7 (Support 40%).

Table 2 Acupoint combination frequency statistics table

No.	Acupoint groups	Frequency
1	百会(DU20) – 内关(PC6)	32
2	百会(DU20) – 太冲(LR3)	31
3	百会(DU20) – 神门(HT7)	29
4	太冲(LR3) – 内关(PC6)	28
5	百会(DU20) – 神庭(EX-HN6)	25
6	太冲(LR3) – 神门(HT7)	24
7	内关(PC6) – 神门(HT7)	24
8	百会(DU20) – 太冲(LR3) – 内关(PC6)	23
9	百会(DU20) – 内关(PC6) – 神门(HT7)	22
10	百会(DU20) – 四神聪(EX-HN1)	20

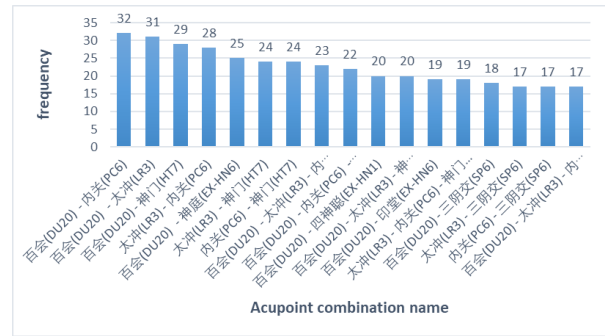


Figure 4 Acupoint combination frequency statistics table

Table 3 Statistical Table of Acupoint Rules

No.	Rule	Confidence
1	印堂(EX-HN6) → 百会(DU20)	0.95
2	内关(PC6), 神门(HT7) → 百会(DU20)	0.916666667
3	太冲(LR3), 内关(PC6), 神门(HT7) → 百会(DU20)	0.894736842
4	神庭(EX-HN6) → 百会(DU20)	0.892857143
5	神门(HT7) → 百会(DU20)	0.852941176
6	百会(DU20), 太冲(LR3), 神门(HT7) → 内关(PC6)	0.85
7	太冲(LR3), 神门(HT7) → 百会(DU20)	0.833333333
8	太冲(LR3), 内关(PC6) → 百会(DU20)	0.821428571
9	内关(PC6) → 百会(DU20)	0.820512821

Note: “→” means that the left and right points often appear as a combination at the same time; the closer the confidence is to 1, the greater the frequency of the combination.

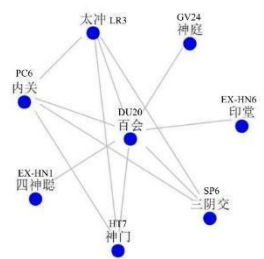


Figure 5 Support is 20%

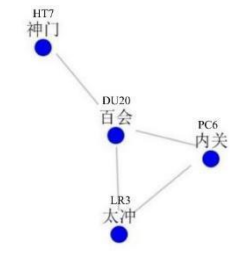


Figure 6 Support is 30%

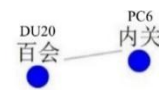


Figure 7 Support is 40%

The network display diagram shows: when the support is (20%) (figure 5), it can fully display the use of acupoints: when the support is 30% (figure 6), the acupuncture points clearly show the formula of the formula, and Baihui (DU20) often It is used in combination with several acupoints; when the support reaches 40% (figure 7), it is clearly obtained that the acupoint with the highest frequency of use with Baihui

(DU20) is Neiguan (PC6) acupoint. It shows that the main acupuncture points in the prescription of acupuncture treatment for post-stroke depression should be Baihui (DU20) and Neiguan (PC6).

Discussion

Post-stroke depression is a concomitant disease of stroke. Its clinical manifestations include hemiplegia, partial numbness, mouth and tongue askew and unsmooth speech, as well as emotional disturbances seen in depression syndrome, manifested as depressed mood, emotional restlessness, irritability and crying, flank pain, etc. [5-6]. Traditional Chinese medicine believes that PSD belongs to the category of "depression syndrome" in Chinese medicine. Clinical treatment is mostly based on the heart and brain, and is closely related to the liver, spleen and kidney [7]. The etiology and pathogenesis of this disease is that qi, blood, phlegm, and fire stasis block the brain collaterals, causing patients to have anxiety and worry, and affecting the liver's dredging function, causing the liver to lose its balance and qi stagnation. Therefore, acupuncture treatment should adjust the qi mechanism and improve the function of the viscera, so as to achieve the effect of promoting qi and relieving depression, calming the heart and soothing the mind [8].

The study found that the top 4 acupoints with the highest frequency (frequency > 30) in the treatment of PSD are listed in table 1 : Baihui (DU20), Taichong (LR3), Neiguan (PC6), Shenmen (HT7). Baihui (DU20) is the meeting point of all yang, the meeting of the three yang meridians of the hands and feet, the Governing Vessel and the liver meridian, located the top of the head. 《Ling Shu • Wei Qi》 says : "The qi in the head has a street, and if the qi is in the head, it stops in the brain." [9] From the perspective of traditional Chinese medicine and meridian theory, "the brain is the home of the primordial spirit", control the spirit, Baihui (DU20) belongs to the Governing Vessel, and the Governing Vessel connects to the brain, it illustrates the close relationship between the brain and the Governing Vessel. Depression is mainly manifested as abnormal emotions, abnormal activities of "spirit", patients often have symptoms such as decreased interest, decreased appetite, and decreased sense of happiness. Baihui (DU20) has the functions of refreshing the brain and opening the orifices, calming the heart and soothing the mind, calming the liver and subduing the yang, regulates the qi of the Governing Vessel, invigorates the yang qi, and has the effect of refreshing the brain and opening the mind, strengthening the brain and calming the mind. Taichong (LR3) is the Shu-stream point and Yuan-source point of the liver meridian, the liver controls emotions, this acupoint has the functions of clearing liver and improving eyesight, calming liver and relieving wind, soothing liver and gallbladder, activating meridians and collaterals. Taichong (LR3) is the Yuan-source point, it is the place where the original

qi of the viscera passes and stays. The main function of this point is to regulate the deficiency and excess of the liver and the liver meridian, so acupuncture at Taichong (LR3) can soothe the liver and purify fire and regulate emotions. Neiguan (PC6) point is the collateral point of the pericardium meridian of Hand Jueyin, where the eight meridians meet. The pericardium acts on behalf of the heart to receive pathogenic, stores the spirit in the heart, controls the blood vessels, and is the master of the internal organs 《Zhen Jiu Jia Yi Jing》 says: "The mind is sullen and good at panic, the heart is sad, and Neiguan (PC6) is the main ". It is said that the human body's consciousness, thinking and other spiritual activities all depend on the commander of the heart. If the function of the mind governing consciousness is abnormal, abnormality of spirit, consciousness and thinking activities can occur. Acupuncture at this point can adjust the mind, regulate qi and blood, promote the circulation of qi and blood, and relieve mental tension. Shenmen (HT7), Shen means God, is opposite to ghosts; Men means door, the door of entry and exit. The name of this acupoint means that the qi and blood of the internal meridians of the heart meridian are connected to the external meridians of the heart meridian. Shenmen (HT7) is the Shu-stream point and the Yuan-source point of the heart meridian of Hand Shaoyin, this acupoint has the functions of calming the heart and calming the mind, nourishing the heart and nourishing qi, and is good at treating mental disorders.

The analysis of the overall correlation law of acupoint allocation for post-stroke depression shows that the common acupoint combinations with high correlation (frequency>25) (see table 2) are Baihui (DU20) - Neiguan (PC6), Baihui (DU20) - Taichong (LR3), Baihui (DU20) - Shenmen (HT7), Taichong (LR3) -Neiguan (PC6). Baihui (DU20) - Neiguan (PC6): Baihui (DU20) belongs to the Governing Vessel, and the Governing Vessel connects to the brain, the brain is the house of the primordial spirit, and has the power to adjust the mind and refresh the brain; Neiguan (PC6) is the collateral point of the pericardium meridian, and the confluence point of the eight meridians connects the Yin Linking Vessel. The heart controls the blood vessels, stores the spirit, and is the master of the internal organs. It has the effect of widening the chest and regulating qi, opening the orifices and relieving depression. Depression is mainly located in the heart, brain, and liver, and the combination of these two points can refresh the brain and open the mind, calm the heart and soothe the mind. Baihui (DU20) belongs to the Governing Vessel, emphasizes the relationship between the Governing Vessel and the brain, and has the effect of conditioning the brain, spirit, and yang qi; Taichong (LR3) belongs to the Yuan-source point of the liver meridian of Foot Jueyin, which can soothe the liver and regulate qi. The two acupoints are used together to regulate the brain and spirit, soothe the liver and gallbladder, and relieve depression. Baihui (DU20) -

Shenmen (HT7): Baihui (DU20) belongs to the Governing Vessel, and the Governing Vessel enters the brain; Shenmen (HT7) is the Shu-stream point and the Yuan-source point of the heart meridian of Hand Shaoyin, the heart stores the spirit, so acupuncture this point can calm the mind, and combined with Baihui (DU20) in the treatment of mental illness, it has a miraculous effect, and has the effect of invigorating depression and refreshing the brain [10]. Taichong (LR3) - Neiguan (PC6): Taichong (LR3) is the Yuan-source point of the liver meridian of Foot Jueyin. The liver controls the emotions and regulates qi. Taichong (LR3) is the key point for soothing liver and regulating qi; Neiguan (PC6) is the collateral point of the pericardium meridian of Hand Shaoyin, which can adjust the power of mind and spirit. The combination of two points has the effect of soothing the liver and regulating qi, calming the heart and soothing the mind.

From the statistical results in table 3, it can be seen that there are 9 items local and distal compatibility such as Neiguan (PC6) - Shenmen (HT7) - Baihui (DU20), Taichong (LR3) - Neiguan (PC6) - Shenmen (HT7) - Baihui (DU20), etc. Two items are mainly based on local acupoint selection, such as Yintang (EX-HN6) - Baihui (DU20) and Shenting (DU24) - Baihui (DU20), which reflects "where the acupoints are, where the indications are"; Because the lesions of post-stroke depression are located in the brain, the etiology and pathogenesis are closely related to the heart and liver. According to the statistical results, it can be seen that the probability of local acupoint selection combined with distal acupoint selection is the highest, indicating that in clinical treatment of post-stroke depression, the combination of local and distal acupoints at the same time is effective, followed by local selection alone has a certain effect.

Conclusion

Traditional Chinese Medicine Inheritance Support System is an important auxiliary tool for data mining and analysis of hidden clinical information laws. The results of acupoint frequency and acupoint compatibility of clinical acupuncture treatment of PSD can be better summarized, which can provide a reference plan for clinical treatment of PSD, which is suitable for physicians to promote and use without complex syndrome differentiation. Of course, the results obtained in this study are all based on data mining technology, and the specific application should also be combined with clinical practice.

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Linalool Exerted a Neuroprotective Activity against Corticosterone-induced Apoptosis of PC12 cells

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ABSTRACT

Introduction: Linalool is an active compound found in many essential oils having sedative, anxiolytic and analgesic properties. Therefore, these essential oils have been usually used in aromatherapy to reduce stress. However, the mechanism underlying anti-stress of linalool has not been investigated.

Objective: To investigate the mechanism of neuroprotective action of linalool on corticosterone-induced neurotoxicity in PC12 cells.

Methods: PC12 cells were treated with 650 μ M of corticosterone in the absence or presence of linalool or fluoxetine for 24 hours. PC12 was treated with corticosterone 650 μ M and difference concentration of linalool (100, 200, 300, 400, 500, 600 and 700 μ M) for 24 hours. Methyl thiazolyl tetrazolium (MTT) assay and lactate dehydrogenase (LDH) detection were investigated to confirm the neuroprotective effect of linalool against cell damage caused by corticosterone. Then, the intracellular Ca^{2+} content was measured by fluorescent labeling.

Results: Linalool 100 and 200 μ M significantly increased cell viability compared with the group treated with corticosterone alone ($p < 0.05$). Linalool 100, 200, 300 and 400 μ M significantly ($p < 0.05$) reduced LDH release. Linalool (100, 200, 300, 400, 500 and 600 μ M) tend to reverse the increased intracellular Ca^{2+} produced by corticosterone.

Conclusion: These findings indicated that linalool exerted a neuroprotective activity against corticosterone-induced neuronal apoptosis in PC12 cells. The mechanism underlying this action may be partly due to the attenuation of intracellular Ca^{2+} overloading under stress conditions. However, further studies need to investigate the precise cellular and molecular mechanisms underlying this neuroprotective activity of linalool.

Keywords: linalool; corticosterone; PC12; apoptosis; intracellular calcium

Introduction

Depression is one of the most common mental disorders, leading to a decline in social ability, disability and many diseases [1,2]. The hypothalamic-pituitary-adrenal (HPA) axis activation is one of the acceptable mechanisms underlying the pathophysiology of depression. Dysregulation of the HPA axis results in elevated cortisol, the stress hormone as well as corticotropin-releasing hormone as well as hippocampal volume reduction [3,4]. Antidepressant drugs such as

fluoxetine can inhibit the activation of the HPA axis [5]. However, long-term treatment of antidepressant can cause many side effects, including nausea, insomnia, agitation, weight gain, somnolence, sexual dysfunction, and cardiovascular adverse events. Therefore, complementary and alternative medicines (CAM) have been increasingly used for treating depression [6]. Aromatherapy, one of the CAM options, has been frequently chosen by patients with depressive symptoms since it is an invasive and inexpensive treatment [7].

Lavender [8,9] and bergamot [10], essential oils commonly used in aromatherapy to reduce depressive symptoms, contain the active components including linalool [11]. The previous study found that linalool demonstrated antidepressant-like effects by decreasing immobility time in the tail suspension test in mice [12]. There are many studies investigating the effect of linalool in various stress conditions. For example, linalool attenuated the decreased cell viability against glucose/serum deprivation (GSD)-induced cytotoxicity, indicating its neuroprotective effect [13]. Recently, Migheli et al (2021) also reported that linalool protected PC12 cells from H₂O₂-induced oxidative stress by increasing cell viability, reducing LDH release and ROS production [14]. However, the neuroprotective action of linalool on stress induced by corticosterone, another stress hormone, has not been investigated. Therefore, the aim of the present study was to investigate the mechanism of neuroprotective action of linalool on corticosterone-induced neurotoxicity in PC12 cells.

Methodology

Cell culture

PC 12 cells were ordered from the American type culture collection (ATCC) (Manassas, USA). PC12 cells were cultured in collagen-coated plate and maintained with Dulbecco's modified Eagle's medium (DMEM) cell culture, consisting of 0.35% glucose, 10% horse serum, 5% fetal bovine serum (FBS) and 100 U/ml kanamycin and subsequently incubated at 37°C for 48 hours until 70% cell growth. Then, the cells were initially incubated with linalool solution plus 650 µM corticosterone for 24 hours and then studied 1) survival of PC12 cells against corticosterone, 2) LDH secretion from PC12 cells, and 3) intracellular calcium level in PC12.

Chemicals

Linalool, fluoxetine and corticosterone were purchased from Sigma–Aldrich (St Louis, MO, USA). Dimethyl sulfoxide (DMSO) was obtained from Millipore (Billerica, MA, USA) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from MP Biomedical (Illkirch, France). *Fura-2/AM* dye was purchased from Invitrogen (Carlsbad, CA, USA).

Cell viability assay

Cell viability was determined by using (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) MTT assay. In brief, PC12 cells were cultured in 96 well plates at a density of 5×10³ cells/well in serum-free DMEM and allowed to adhere at 37 °C, 5%

CO₂ incubator for 24 hours. The cells were co-treated with various concentrations of linalool (100, 200, 300, 400, 500, 600, 700 µM) or 10 µM fluoxetine and 650 µM corticosterone and incubated for 24 hours. Then, a 20 µl MTT solution was added and the plate was further incubated in a CO₂ incubator at 37 °C for 4 hours. The medium was aspirated off and 100 µL DMSO was added to dissolve formazan crystals. The absorbance of the purple solution was measured at 545 nm by spectrophotometer (Epoch™ microplate spectrophotometer; Biotek, Winooski, VT, United States). Cell viability was shown as a percentage of non-treated control.

Lactate Dehydrogenase (LDH) Assay

This assay is based on the measurement of LDH release from the cells due to damage of plasma membrane. Thus, the presence of stable LDH in cell culture medium can be detected and used as an indicator of cellular toxicity. LDH release was measured by following the instruction manual of LDH activity assay kit and using spectrophotometer (Epoch™ microplate spectrophotometer; Biotek, Winooski, VT, United States). All experiments were performed in triplicate.

Measurement of Intracellular Ca²⁺ Concentration

Quantitative intracellular calcium was determined using *Fura-2/AM*, fluorescence indicator of free calcium. Initially, PC12 cells were co-treated with various concentrations of linalool (100, 200, 300, 400, 500, 600, 700 µM) or 10 µM fluoxetine and 650 µM corticosterone and incubated for 24 hours. Then, the cells were collected and incubated with *Fura-2/AM* and pluronic F-127 for 45 min at 37 °C. After washing with ice-cold PBS solution, the fluorescence was measured by microplate reader (Epoch™ microplate spectrophotometer; Biotek, Winooski, VT, United States) at 400 nm excitation and 505 nm emission. Cell viability was shown as a percentage of non-treated control.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The statistical analysis was performed with one-way ANOVA followed by Newman-Keuls multiple comparison test using GraphPad Prism 9 software. The differences between mean values were accepted as statistically significant at $p \leq 0.05$. Each experimental group consisted of 6 cell samples.

Results

Effect of linalool on viability of PC12 cells after inactivation by corticosterone

As shown in figure 1, 650 μM corticosterone significantly decreased cell viability of PC12 ($p < 0.01$). The 100 and 200 μM linalool significantly attenuated the decreased cell viability produced by corticosterone ($p < 0.05$), while fluoxetine 10 μM resulted in less cell survival than incubating with corticosterone alone ($p < 0.01$).

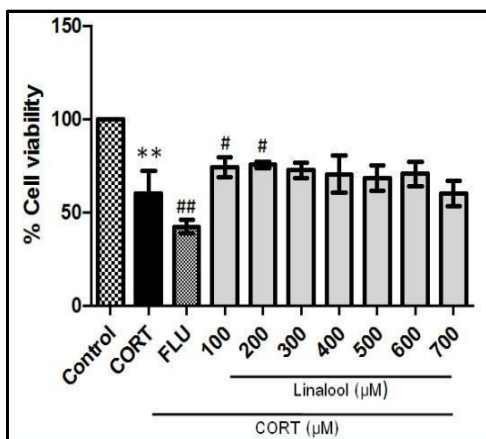


Figure 1 Effects of linalool on corticosterone (CORT)-induced cytotoxicity of PC12 cells measured by MTT assay (mean \pm SD, n= 4-6). ** $p < 0.001$ as compared with control (CONT) group; # $p < 0.05$ and ## $p < 0.01$ as compared with the CORT group.

Effect of linalool on corticosterone-induced LDH release from PC12 cells

As indicated in figure 2, the percentage of LDH release was significantly increased for corticosterone-treated PC12 cells ($p < 0.001$). Linalool (100, 200, 300 and 400 μM) but not 10 μM fluoxetine significantly attenuated the increased LDH release produced by corticosterone ($p < 0.01$).

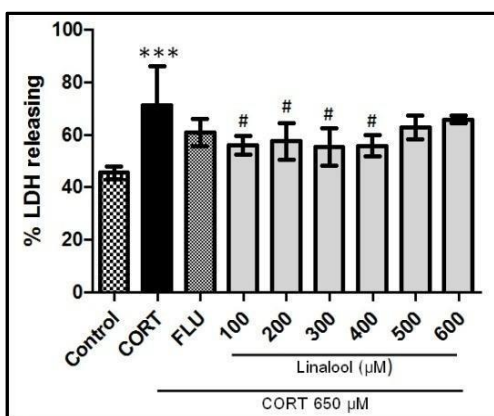


Figure 2 Effects of linalool vetiver oil on LDH release in corticosterone (CORT)-induced PC12 cells (mean \pm SD, n= 4-6). *** $p < 0.001$ as compared with control (CONT) group; # $p < 0.05$ as compared with the CORT group.

Effect of linalool on corticosterone-induced intracellular Ca^{2+} concentration in PC12 cells

As shown in figure 3, after treatment of PC12 cells with 650 μM corticosterone for 24 h, the concentration of intracellular Ca^{2+} markedly increased as compared with the control group ($p < 0.01$). By contrast, linalool (100, 200, 300, 400, 500 and 600 μM) tend to reverse the increased intracellular Ca^{2+} produced by corticosterone. Surprisingly, 10 μM fluoxetine did not attenuate the increased Ca^{2+} release produced by corticosterone ($p < 0.01$).

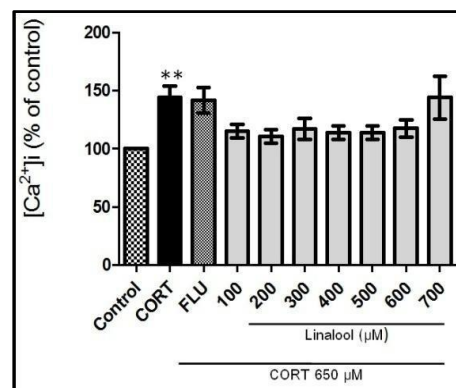


Figure 3 Effects of linalool on corticosterone-induced intracellular Ca^{2+} concentration in PC12 cells (mean \pm SD, n= 4-6). ** $p < 0.01$ as compared with the control (CONT) group.

Discussion

Linalool is the principal component of many essential oils produced from aromatic plants including members of the Lamiaceae (mints), Lauraceae (laurels, cinnamon, rosewood) and Rutaceae (citrus) [15-17]. Linalool, a lipophilic monoterpene, may pass the blood brain barrier to exert effects on brain function. Many studies have demonstrated that linalool exerts neuroprotective, anti-inflammatory, and antioxidant effects in the brain [18-21]. Linalool demonstrated antidepressant-like effects in animal models and neuroprotective action against glucose/serum deprivation and H_2O_2 induced oxidative stress. However, the neuroprotective action of linalool on stress induced by corticosterone, another stress hormone, has not been investigated. These findings indicated for the first time that linalool exerted a neuroprotective activity against corticosterone-induced neuronal apoptosis in PC12 cells. The previous studies have shown that high corticosterone level caused severe PC12 damage including apoptosis and decreased nerve growth factor (NGF) mRNA levels in cells [22], but antidepressants can alleviate this damage [23]. This is consistent with the present study that showed that linalool attenuated the decreased cell viability of PC12 induced by corticosterone. Unexpectedly, fluoxetine, the standard

antidepressant, potentiated the decreased cell viability produced by corticosterone. Similarly, after fluoxetine, the cell viability showed a significant increase at day 1 but a slight decrease at day 2 [24]. Recently, it was also shown that neuronal death was markedly increased with treatment of 10-30 μ M fluoxetine [25]. Therefore, our results suggest that linalool may protect PC12 cells from the toxic effect of the hormone corticosterone superior to fluoxetine, used with high dose or longer duration. LDH is an enzyme present in the cytoplasm of all mammalian cells. Normally, the plasma membrane does not allow the secretion of LDH, but LDH is released into the extracellular fluid following cell damage. Thus, the LDH leakage has been commonly used as the marker of cell damage or injury. The previous study showed that antidepressant medications can attenuate PC12 cell damage from corticosterone by reducing LDH release [26]. Consistent with this study, it was found that high corticosterone level increased LDH secretion, likely due to cell damage as shown in the MTT experiment with reduced cell viability. In contrast, linalool attenuated the increased LDH secretion and decreased cell viability induced by corticosterone treatment. Thus, this study suggests that linalool has a protective effect on PC12 cell damage from corticosterone exposure, while fluoxetine does not have a protective effect against cell damage from this stress hormone. Intracellular calcium is a key signaling ion involved in neuronal cell function including synaptic activity, cell communication and adhesion [27]. After corticosterone exposure, intracellular calcium was elevated, resulting in increased LDH release and ROS production which subsequently cell damage [28]. This study also found that the concentration of intracellular Ca^{2+} was upregulated in corticosterone-treated PC12 cells but slightly downregulated in the cells co-treated with linalool.

Conclusion

These results indicate that the neuroprotective effect of linalool against corticosterone-induced apoptosis may be partly mediated by the inhibition of intracellular Ca^{2+} concentration intracellular calcium overload. However, further studies need to investigate the precise cellular and molecular mechanisms underlying this neuroprotective activity of linalool.

Competing Interests

No conflicts of interest, financial or otherwise, are declared by the authors.

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Author contributions

All authors contributed to the final version of the manuscript.

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Anti-biofilm Potential of *Boesenbergia rotunda* L. Extract and Synergy with Cloxacillin on Biofilms of β -lactam-resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: A biofilm is extracellular substances produced by microorganisms to protect themselves from harmful agents, including antibiotics. Bacteria living in biofilms are known to have higher resistance to antimicrobial therapy and is one of the most important mechanisms of resistance to antibiotics. Hence, development of a novel anti-biofilm agent is of far-reaching importance.

Objective: To investigate the anti-biofilm effect of *B. rotunda* extract (BRE) and its synergism with cloxacillin (CLX) on biofilms of β -lactam-resistant *Staphylococcus aureus*.

Methods: *B. rotunda* extract was carried out by macerating with 99.9% ethanol. The extracts were subsequently dried using a rotary evaporator and lyophilized. The anti-biofilm activity and synergistic effect of BRE were carried out by a crystal violet assay. Quantitation of biofilms were done by a spectrophotometer, while qualitative analysis of biofilm formation was observed under a light microscope.

Results: Following 24 h of treatment, the result from quantitative analysis of biofilm formation using a crystal violet assay revealed that BRE significantly eradicated biofilms of β -lactam-resistant *S. aureus*. Interestingly, the combination of BRE and CLX synergistically inhibited biofilm formation. In line with qualitative analysis of biofilm formation, bacteria treated with BRE and CLX, either alone or in combination, had explicitly lower biofilm than untreated control.

Conclusion: BRE potentially destroyed biofilms and had synergistic effect with CLX in eradication of biofilms of β -lactam-resistant *S. aureus*. These findings from the evidence that the combination of BRE and CLX could be a good candidate for further development as a novel agent for treatment of recalcitrant infection caused by β -lactam-resistant *S. aureus*.

Keywords: *Boesenbergia rotunda* extract; biofilms; synergism; *Staphylococcus aureus*

Introduction

A biofilm is a structured community of bacteria embedded in a self-produced extracellular polymeric substance (EPS) composing of polysaccharides, proteins, nucleic acids, lipids, and humorous-like substances [1]. This matrix makes it difficult to eradicate and causes chronicity of infection [2]. Biofilms are formed on many implanted medical devices, such as central venous catheters, heart valves, urinary catheters, orthopedic devices and even contact lenses [3,4]. The bacteria living in a biofilm can become

as much as 10-1000 times more resistant to antimicrobial agents compared with normally seen in planktonic cells [3]. Failed penetration of the agents, delayed diffusion of antibiotic, and slow-growing or non-growing state are the contributing factors for resistance to antibiotics [5]. Accumulation of β -lactamase with an increased upregulation of efflux pumps allows bacteria to survive in the biofilm [6]. Treatment of bacteria in the biofilm with antibiotics often fails to kill. Currently available antibiotics are

most effective against planktonic bacteria, but they are typically ineffective to eradicate bacterial biofilm and persistent infections. So, this indicates a necessity to research and develop novel agents targeting biofilms [7]. Plant-derived antibiofilm agents are of interest. Flavonoids isolated from *Moringa oleifera* seed extract had inhibitory effects on biofilms of *S. aureus*, *P. aeruginosa* and *C. albicans* [8]. Salvisipone extracted from hairy roots of *Salvia sclarea* L. also showed antibiofilm activity against *S. aureus* and *S. epidermidis* [9]. In addition, phytochemical/antibiotic or antibiotic/antibiotic combinations are a promising approach to eradicate bacterial biofilms. Coelho and Pereira (2013) also found the anti-biofilm activity of three essential oils, including cinnamon (*Cinnamomum zeylanicum*), tea tree (*Melaleuca alternifolia*) and palmarosa (*Cymbopogon martini*), either used singly or in combination with ciprofloxacin. They found that *P. aeruginosa* biofilms were inhibited by each of these essential oils [10]. N-acetylcysteine, EDTA, ethanol and talactoferrin (TLF) also revealed synergy with some antibiotics against *S. epidermidis* and *C. albicans* biofilms, which are common catheters-indwelling pathogens. These combinations were proposed to be new regimens for treatment of infected catheters [11]. *Staphylococcus aureus* is a Gram-positive coccus belonging to the family *Staphylococcaceae*. It is considered as the normal microbiota of human mucous, nasopharynx and skin. This bacterium is among the greatest threats to human health in both community onset and healthcare-acquired infections. It causes a variety of human illnesses, including pneumonia, meningitis, toxic shock syndrome, bacteremia, wound sepsis, osteomyelitis, and endocarditis [12]. Methicillin-resistant *S. aureus* (MRSA) was recorded in the year of the drug's launch. Since then, MRSA has become one of the most serious public health problems worldwide. Using a combination approach of a phytochemical and antibiotic is a promising avenue for combating multidrug-resistant bacteria and their biofilms. *B. rotunda*, or locally known in Thai as Kra-chai khaw, has been used as traditional ethnomedicine for treating several human ailments including rheumatism, muscle ache, fever, gouty arthritis, bowel disorder, abdominal distension, carminative, appetite promotion, gastric disturbances, anti-ulcer, anti-inflammation, antioxidant, anti-viral, anti-parasite and anticancer. Interestingly, this herbal plant exhibits strong antibacterial activity with a wide spectrum against pathogenic bacteria [13]. Due to scarce data, the present study therefore investigated the anti-biofilm activity and synergy effect of *B. rotunda* extract (BRE) with cloxacillin (CLX) against biofilms of β -lactam-resistant *S. aureus*.

Methodology

Bacterial strains and antibiotic

β -lactam-resistant *S. aureus* DMST 20651 was obtained from the Department of Medical Science,

Ministry of Public Health, Thailand. Cloxacillin antibiotic ($\geq 95\%$ purity) was purchased from Sigma-Aldrich, Singapore.

Extraction of B. rotunda

Rhizomes of *B. rotunda* were domestically obtained from Nakhon Ratchasima province, Thailand during August 2014. The plant sample was authenticated by comparing with the Forest Herbarium specimen (a voucher specimen BKF No. 192160). Finely ground of dried rhizome were macerated with 99.9% ethanol for a week at room temperature. The extracts were then filtered through Whatman no. 1 filter paper before removing the solvent using a rotary evaporator under reduced pressure. Subsequently, the extracts were dried by a lyophilized [14].

Quantitation of bacterial biofilm

The inhibitory effect of BRE either alone or in combination on biofilm development were evaluated as previously described by Gopal et al. (2014) with some modifications [15]. Briefly, an 18-h culture of test bacteria was adjusted in saline to give 5×10^6 CFU/mL. Twenty microlites of adjusted bacterial suspension were added to 190 μ L of broth medium supplemented with 0.2% glucose plus BRE, or antibiotic alone, or in combination. The concentration at half-MIC or FIC of BRE or antibiotic from a previous study was employed [14]. Following incubation at 37 °C for 48 h, the culture medium was discarded, and free-floating planktonic cells were washed with distilled water. The adhered biofilms were stained with 0.4% (w/v) crystal violet solution for 30 min. The excess stain was washed off thoroughly with distilled water. After the wells are air-dried, the stained biofilms were then solubilized with 100% ethanol before measuring an absorbance using a microplate reader at 595 nm. The optical density values represented adhering bacteria and forming a biofilm

Microscopic analysis of bacterial biofilm

The light microscope analysis of bacterial biofilm was examined following the method of Nithya and Pandian (2010) and Packiavathy et al (2014) with few modifications [16,17]. Two hundred microliters of adjusted bacteria (5×10^6 CFU/mL) were added to 1800 μ L of fresh medium (supplemented with 0.2% glucose) containing BRE either alone or in combination with antibiotics in a 24-well polystyrene plate. Wells with and without test bacteria were used as controls. The plate was incubated at 37 °C for 48 h. Afterwards, the planktonic cells were removed prior to staining the adhered with 0.4% crystal violet solution. The excess crystal violet was washed off by repeatedly submerging the plate into reservoirs of distilled water. The plate was then placed in an incubator at 37 °C until dry. The stained biofilm was viewed under a light microscope.

Statistical analysis

Data were expressed as mean \pm standard error of the mean (SEM) from 6 repetitions. The results were analyzed and compared by One-way ANOVA followed by Tukey's HSD Post-hoc test. The different alphabetical characters represent a statistically significant difference between each group ($p < 0.01$).

Results

Ethanollic extraction of *B. rotunda* yielded $10.3 \pm 0.94\%$ (w/w). The antibiofilm activity of CLX and BRE either used singly or in combination against MRSA DMST 20651 using a standard quantitative biofilm assay is demonstrated in figure 1. After 48 of treatment, MRSA DMST 20651 grown in the absence of any antimicrobial agent showed the highest biofilm biomass. All treated groups significantly dislodged the biofilm of MRSA DMST 20651 compared with a control group, $p \leq 0.01$. CLX (256 $\mu\text{g}/\text{mL}$)-treated group exhibited lowest biofilm formation, but no significant difference compared with BRE (8 $\mu\text{g}/\text{mL}$) alone and BRE (8 $\mu\text{g}/\text{mL}$) plus CLX (1 $\mu\text{g}/\text{mL}$), $p > 0.01$.

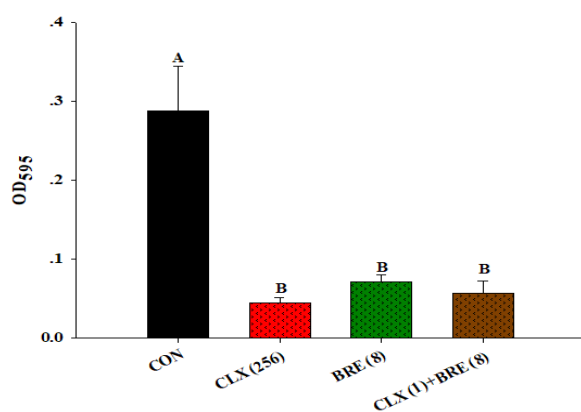


Figure 1 Quantitative analysis of biofilm formation of MRSA 20651 after exposure to no antibacterial agents, *B. rotunda* extract and cloxacillin either used alone or in combination. CON = control, CLX (256) = cloxacillin at a concentration of 256 $\mu\text{g}/\text{mL}$, BRE (8) = *B. rotunda* [18] (8) = cloxacillin at a concentration of 1 $\mu\text{g}/\text{mL}$ and *B. rotunda* extract at a concentration of 8 $\mu\text{g}/\text{mL}$.

The effect of 256 $\mu\text{g}/\text{mL}$ CLX, 8 $\mu\text{g}/\text{mL}$ BRE alone and the combination of 1 $\mu\text{g}/\text{mL}$ CLX plus 8 $\mu\text{g}/\text{mL}$ BRE on biofilm formation of MRSA 20651 were also qualitatively examined under a light microscope. The results of this assay are shown in figure 2. The crystal violet staining in a control group exhibited a thick coating of biofilm (figure 2A), whereas the explicit reduction in biofilm formation was observed in cells treated with BRE and CLX either alone or in combination. The CLX-treated group showed the greatest activity in the eradication of biofilm formation compared with BRE alone and BRE plus CLX. MRSA DMST 20651 cells were observed under light microscopy.

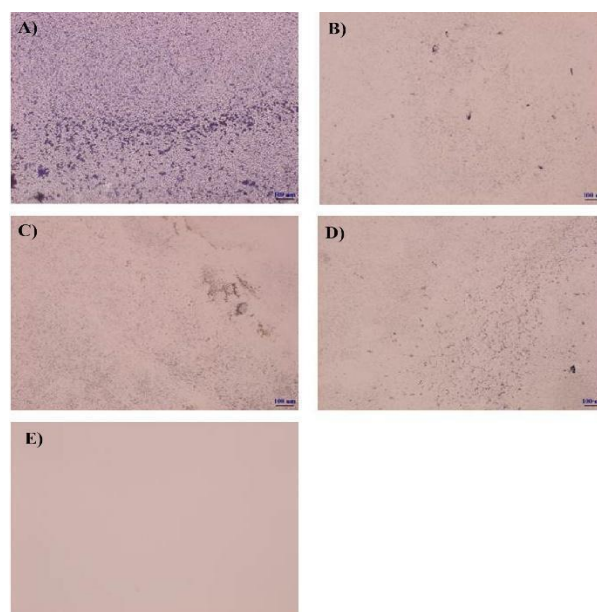


Figure 2 Qualitative microscopic analysis of biofilm formation using a light microscope. A = positive control, B = CLX (256 $\mu\text{g}/\text{mL}$)-treated group, C = BRE (8 $\mu\text{g}/\text{mL}$), D = CLX (1 $\mu\text{g}/\text{mL}$) plus BRE (8 $\mu\text{g}/\text{mL}$)-treated group, E = a negative control. Bar = 100 μm and magnification = 400x

Discussion

A biofilm is known to be problematic for several human ailments. Bacteria that live in a biofilm present high resistance to antibiotics leading to treatment failure. This is due to biofilms being highly resistant to immune killing and clearance, and to treatment with antimicrobial agents [19]. Bacterial biofilm can form on either environmental abiotic surfaces or biotic surfaces. The biofilm-formed cultures of *Actinomyces pyogenes*, *Corynebacterium renale*, *Corynebacterium pseudotuberculosis*, *S. aureus*, *Staphylococcus hyicus* and *Streptococcus agalactiae* in biofilm were resistant to ampicillin, ceftiofur, cloxacillin, oxytetracycline, penicillin G, streptomycin, tetracycline, enrofloxacin, and erythromycin. Whereas, all of these antibiotics were effective against those strains in planktonic cells [20]. Based on our previous study, β -lactam-resistant *S. aureus* used in the present study was highly resistant to a number of antibiotics including cloxacillin and ampicillin and cefazolin. Regarding anti-biofilm activity of BRE [14], this study is the first to show that BRE either alone or in combination with CLX prevents the development of biofilms of β -lactam-resistant *S. aureus*. The MRSA DMST 20651 biofilm was significantly minimized following exposure to BRE either used alone or in combination. This finding agrees with Yanti and colleagues who reported that Panduratin A isolated from *Kaempferia pandurata* Roxb (synonym *B. rotunda* extract) has the ability to disperse biofilm formed by multispecies oral bacteria [18]. Also, flavonoids from *Moringa oleifera* seed extract have

inhibitory effects on biofilms of *S. aureus*, *P. aeruginosa* and *C. albicans* [21]. In addition, other flavonoids, such as naringenin, kaempferol, quercetin, and apigenin have also been reported to have the ability to eradicate *Vibrio harveyi* and *E. coli* biofilms [22].

Conclusion

BRE possesses anti-biofilm activity and has a synergistic effect by potentiating CLX to eradicate bacterial biofilms of β -lactam-resistant *S. aureus*. The combination of BRE and CLX could be an attractive candidate of a novel pharmaceutical adjuvant for treatment of life-threatening β -lactam-resistant *S. aureus*.

Competing Interests

The authors have declared no conflict of interests.

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Author contributions

YT and GE conceived and conceptualized this project. PK and YT carried out experiments as well as analyzed data.

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Psychological Effects of Nail Color

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ABSTRACT

Introduction: Nail polish is a cosmetic used to apply color for beautifying fingernails and toenails. Nail polish may be provided benefits as color therapy for balancing psychological problems.

Objective: The objective of this study was to find the benefit of colors from nail polish application based on the theory of color therapy.

Methods: The participants were 200 female students at Mae Fah Luang University. They were divided into 2 groups; blinded and unblinded by their perception of color therapy. The participants applied their nails with different colors based on their emotions and determined the color effects after 1 week of application by answering the questionnaires.

Results: The results found that the main problems of participants were depression (13.00%), rough (12.50%), complicated (11.50%), and lack of confidence (10.50%), respectively. The score of the emotion of each participant was determined before and after nail polish application and found a significant decrease ($p < 0.05$) in the emotion problem after the experiment. State-Trait Anxiety Inventory (STAI) and Beck Anxiety Inventory (BAI) were used to measure anxiety levels and found a significant decrease ($p < 0.05$) in both scores after nail polish application. The positive psychological effects of nail color were found in both groups without any bias in the results. In addition, most of the participants were satisfied with their nail colors and half of them felt a change in their emotions after nail polish application.

Conclusion: Nail color applications help to improve the emotion and decrease the anxiety levels of participants.

Keywords: Anxiety; Color therapy; Emotions; Nail polish; Psychological

Introduction

Anxiety is a universal human emotion defined as a displeasing feeling of uneasiness, nervousness, apprehension, fear, concern, or worry [1]. There are two main symptoms of anxiety; emotional sensation and physical sensation. Anxiety affects emotional sensations such as nervousness, worry, irritability, self-consciousness, and fear. Anxiety might be leading to physical sensations such as headache, nausea, sweating, hot flashes or chills, vomiting, and increasing blood pressure [2]. Anxiety affects one-eighth of the total population worldwide and has become a significant area of research interest in psychopharmacology [3]. There are many ways to reduce anxiety such as relaxation techniques, herbal medicine, yoga, massages, hypnosis, chiropractic, spiritual healing by others, folk remedies, homeopathy and psychological treatments [4].

Color therapy is one type of psychological treatment. Color therapy is the use of vibration frequency of the spectrum to correct imbalance or disharmony in the human body. The brain perceives these different wavelengths as different colors then the eyes will translate the energy of the light into the nervous impulse, which the brain then interprets as color. These energies outside not only visible but also conscious color vision affect both mind and body which can have an effect on the physical, mental and emotional state [5]. Color therapy can be used for balancing or preventing psychological problems. The impact of color on mood is widely recognized. It is also considered a holistic therapy that activates the body's healing process.

Color may influence a range of psychological, physiological, and behavioral responses [6]. For

example, blue is the intellect color which may improve cognitive performance, consciousness and intellectual. Red is the color of energy that is associated with movement and excitement. Yellow is the color of confidence. Pink is the color of romance. Orange is the color of joy, fun, and happiness. Green is a color of calm. Black is a color of security. Brown is a color of stability. Violet is a color of authenticity and truth. Grey is a color of neutrality and balance [7].

Nail polish is a cosmetic used to apply to human fingernails and toenails to protect and beautify nails. Nail polish consists of lacquer combined with color pigments and several other components, depending on the brand. Nail polish became one of the necessities in women's color cosmetics by the 1940s [8]. Nowadays, nail polish is receiving more attention among consumers. There are various factors for nail polish selection. These factors are color, trend, season, application, and age of consumers [9]. Color is one of the most important factors that affect nail polish selection. Consumers may focus more on color than brand.

The objective of this study was to find the benefit of color nail polish based on the theory of color therapy. The participants applied their nails with different colors based on their emotional problems and determined the color effects by answering the questionnaire. The result from this study might be useful for further use in advertising and marketing nail polish.

Methodology

Materials

Nail polishes were purchased from Yves Rocher Co., Ltd. The colors of nail polishes were yellow, red, pink, blue, orange, green, black, brown, violet, and grey.

Participants

The participants in this study consisted of 200 female students in Mae Fah Luang University, aged 18-30 years who have no history of any dermatological diseases or nail polish allergies.

The participants were divided into 2 groups, 100 participants per group. Group 1 was a blinded group who did not know about the psychological effects of color, and group 2 was unblinded group who know about the psychological effects of color.

This study was approved by the Ethics in Human Research Committee of Mae Fah Luang University (No. REH-59093). All volunteers received information about the study and signed an informed consent sheet.

Research instrument design

A questionnaire was used to study the effects of nail colors on participants before and after nail polish application. The questionnaire was divided into three parts;

Part 1: Compose the general information of participants such as age, education level, school, frequency of nail polish application, knowledge about color therapy, and factors to select nail polish colors.

Part 2: Compose emotional problems of participants, problem level, feelings, and satisfaction before and after nail polish application.

The emotional problem of each participant was used as criteria for selecting the nail color and shown in table 1.

Table 1 The relationship between psychological problems and color therapy [7].

Emotional problems	Color
Lack of confidence	Yellow
Depressed	Red
Rough	Pink
Complicated	Blue
Solitary	Orange
Anxious	Green
Suspicious	Black
Weak	Brown
Confused	Violet
No problem	Grey

The satisfaction level before and after the nail polish application has 6-points Likert scale. The criteria interpretations for the satisfaction level was 6 levels as following; 5.19-6.00 (very high), 4.35-5.18 (high), 3.51-4.34 (Medium), 2.68-3.50 (low), 1.84-2.67 (very low), 1.00-1.83 (dissatisfied).

Part 3: Compose the State-Trait Anxiety Inventory (STAI) [10] and Beck Anxiety Inventory (BAI) [11].

The participants were asked to complete STAI and BAI before and after the nail polish application.

STAI is a self-report to measure the presence and severity of emotional symptoms of anxiety. The STAI is composed of 40 items for measuring State Anxiety (S-Anxiety) and Trait Anxiety (T-Anxiety). S-Anxiety is the intensity of the current feeling composed of 20 items for assessing tension, nervousness, worry, apprehension, and activation or arousal of the autonomic nervous system. T-Anxiety is the relatively stable aspect of anxiety proneness composed of 20 items including general states of calmness, confidence, and security [12].

BAI consists of 21 self-reported items (4-points scale) used to assess the intensity of physical and cognitive anxiety symptoms during the past week while minimizing their relationship with depression. The measurement includes assessment of symptoms such as nervousness, dizziness, and inability to relax. Scores may range from 0 to 63: minimal anxiety levels (0-7), mild anxiety (8-15), moderate anxiety (16-25), and severe anxiety (26-63) [12].

Data analysis

The results such as mean, frequency, standard deviations, and percentage were calculated by Microsoft excel 2010. The statistical analysis was determined by the independent-samples T-test and paired-samples T-

test via the program IBM SPSS statistics version 21 (Trial version).

Results

Emotional problems

The survey about emotional problems found that 33% of group 1 and 18% of group 2 did not have emotional problems. The participants in this group applied their nails with a grey color. However, 67% of

group 1 and 82% of group 2 had emotional problems as shown in table 2. Most of the participants in group 1 felt complicated (13%) and participants in group 2 felt depressed (16%). In addition, the main problems of participants were depression (26 participants, 13%), rough (25 participants, 12.50%), complicated (23 participants, 11.50%), and lack of confidence (21 participants, 10.50%), respectively.

Table 2 Emotional problems of participants.

Emotional problems	Group 1 (%)	Group 2 (%)	Total n (%)
Depressed	10	16	26 (13.00)
Rough	10	15	25 (12.50)
Complicated	13	10	23 (11.50)
Lack of confidence	11	10	21 (10.50)
Confused	6	12	18 (9.00)
Anxious	8	6	14 (7.00)
Solitary	6	5	11 (5.50)
Weak	1	7	8 (4.00)
Suspicious	2	1	3 (1.50)
No problem	33	18	51 (25.50)

The score of emotional problems before and after nail polish application

The level of emotional problems was examined before and after the nail polish application. Table 3 showed that after 1 week of nail polish application, the participants in both groups had significantly decreased their emotional problems scores ($p < 0.05$). The participants of group 1 showed a significant decrease ($p < 0.05$) in problems such as depression, roughness,

complicated, lack of confidence, and anxiety after nail polish application. In group 2, the participants showed a significant decrease ($p < 0.05$) in problems such as depression, roughness, confusion, anxiety, and weakness after nail polish application. In addition, the participant in group 1 felt significantly reduced ($p < 0.05$) in their depression emotion more than the participants in group 2.

Table 3 Score of emotional problems before and after nail polish application

Problem	Group 1 (Blinded)			Group 2 (Unblinded)		
	Before	After	Δ Change	Before	After	Δ Change
Depressed	3.80±1.03	1.80±0.79*	2.00±0.24 ^a	3.56±0.73	2.19±0.98*	1.38±0.25
Rough	3.90±0.99	2.30±0.82*	1.60±0.17	3.67±0.90	2.47±0.64*	1.20±0.26
Complicated	3.92±0.95	3.31±0.75*	0.62±0.20	3.20±0.92	2.60±1.26	0.60±0.35
Lack of confidence	3.18±0.60	2.18±0.75*	1.00±0.15	3.20±1.40	2.30±1.25	0.90±0.15
Confused	3.83±0.98	3.17±1.17	0.67±0.19	3.17±0.94	2.67±1.23*	0.50±0.29
Anxious	3.50±1.31	2.25±1.04*	1.25±0.27	3.67±0.52	2.50±0.84*	1.17±0.32
Solitary	3.33±0.52	3.33±0.52	0.00±0.00	3.20±0.45	2.60±1.52	0.60±1.07
Weak	3.00±0.00	3.00±0.00	0.00±0.00	3.43±0.79	2.00±1.54*	1.43±0.37
Suspicious	2.50±0.71	1.50±0.71	1.00±0.00	3.00±0.00	2.00±0.00	1.00±0.00

* Significant change ($p < 0.05$) when compared with before nail polish application

^a Significant change ($p < 0.05$) when compared with group 2. Data expressed as mean ± S.D.

The score of anxiety levels before and after nail polish application

The results in table 4 showed the anxiety level before and after nail polish application. The scores were determined by using the questionnaire of STAI and BAI. The results found that STAI scores were significantly reduced after nail polish application in both groups ($p < 0.05$) while BAI scores were also reduced in both groups but significantly reduced in group 2 ($p < 0.05$).

S-Anxiety were decreased from 42.08±7.03 to 40.42±5.52 in group 1 and decreased from 44.15±5.68 to 41.73±5.29 in group 2. T-Anxiety were decreased from 43.86±6.23 to 43.80±5.19 in group 1 and decreased from 45.37±5.30 to 44.12±3.91 in group 2.

BAI score was reduced from 24.01±8.60 to 23.04±6.96 in group 1 and significantly reduced from 24.00±7.56 to 21.63±7.25 in group 2.

Table 4 The score of anxiety levels before and after nail polish application

Parameter	Group 1 (Blinded)			Group 2 (Unblinded)		
	Before	After	Δ Change	Before	After	Δ Change
STAI	85.94±12.37	84.12±9.70*	1.82±9.05	89.11±10.80	85.54±8.67*	3.61±8.76
S-Anxiety	42.08±7.03	40.42±5.52	1.70±5.52	44.15±5.68	41.73±5.29	0.20±5.17
T-Anxiety	43.86±6.23	43.80±5.19	2.57±5.27	45.37±5.30	44.12±3.91	1.34±4.48
BAI	24.01±8.60	23.04±6.96	0.99±5.60	24.00±7.56	21.63±7.25*	2.37±4.92

*significant change (p<0.05) when compared with before nail polish application, Data expressed as mean ± S.D. (n=100). STAI = State-Trait Anxiety Inventory, BAI = Beck Anxiety Inventory.

Satisfaction with nail color

The participants rated themselves for satisfaction with their nail color. The results are shown in table 5 that most of them (73% of group 1, 75% of group 2) were satisfied with their nail color. Moreover, half of them (51.00% of group 1, 55.00% of group 2) felt changed in their emotions after the nail polish application.

In addition, the relationship between satisfaction with nail colors and emotional change after nail polish application was determined by the Chi-square test. It was found that there was no relationship between satisfaction with nail colors and emotional changes after nail polish application in both groups (table 6).

Table 5 Satisfaction on nail color

Category	Group 1 (n=100)	Group 2 (n=100)
Satisfaction on nail color		
Satisfied	73	75
Dissatisfied	27	25
Change of emotional after test		
Changed	51	55
Unchanged	30	20
Uncertain	19	25

Table 6 The relationship between satisfaction with nail color and emotional change after nail polish application

		Change of problem after nail polish application			Chi-square	Asymp. Sig. (2-sided)
		Changed	Unchanged	Uncertain		
Group 1	Satisfied	47.95%	32.87%	19.18%	1.987	0.370
	Dissatisfied	59.26%	18.52%	22.22%		
Group 2	Satisfied	50.67%	24.00%	25.33%	3.438	0.179
	Dissatisfied	68.00%	8.00%	24.00%		

Overall satisfaction before and after the experiment

The participants were asked about their satisfaction before and after the experiment. The results in table 7 found that before nail polish application their satisfaction was at a low level but increase to a medium

level after nail polish application in both groups. It was indicated that the nail polish application helped to improve the emotion of participants and increased their satisfaction with the experiment.

Table 7 Satisfaction of participants before and after the experiment

Satisfaction	Group 1	Level of satisfaction	Group 2	Level of satisfaction
Before	3.46±1.27	Low	3.34±1.15	Low
After	4.08±1.21	Medium	4.38±0.96	Medium
Δ Change	0.62±0.06	-	1.04±0.19	-

Discussion

It has been reported that university students had a great deal created anxiety, especially in their study process. There are many factors that lead to an increase in their anxiety such as difficult subjects, a new environment, and relationship problems [13]. Previous studies have suggested that anxiety disorders are more

prevalent among women than men [14]. The data from the emotional problems were used as criteria for choosing the color of nail polish for participants. It was found that most participants felt depressed. The participants who felt depressed applied their nails with red color because it is the color of energy. The previous studies found that a red color could improve depression

due to the red color is very stimulating, exciting, and amplifying the emotion [5]. The previous studies found that a red room raises a room's energy level, stirs up excitement, stimulates conversation, and creates a strong impression [15]. The participants who felt rough applied their nails with pink color to enhance romance, love, and gentle feeling. The participants who felt complicated applied their nails with a blue color to promote intellectuality and enhance performance on cognitive. The computer color screen studies have shown that blue color could enhance the performance of creative and cognitive [16]. The participants who had a lack of confidence applied their nails with yellow color to increase their confidence. The participants who felt confused applied their nails with a violet color which was associated with authenticity and truth. The participants who felt solitary applied their nails with orange color which is the color that combined the energy of red and the happiness of yellow. It is associated with joy, fun, happy and energetic days. The participants who felt anxious applied their nails with green color to balance and harmonize their mind, body, and emotion. It assists in decision-making and creating a sense of calm. The participants who felt suspicious applied their nails with black color which reported as the color of security, emotional safety and evoke strong emotion. The participants who felt weak applied their nails with a brown color associated with stability [7]. Moreover, when comparing the change values, it was found that the participants in group 1 (blinded) were decreased in depression more than in group 2 (unblinded). This finding was different from the previous study in that unblinded group tended to be biased toward the beneficial effects of the study [17]. It can be illustrated that color is able to improve emotional problems without a bias in the results.

The anxiety level before and after nail polish application were determined by using the questionnaire of STAI and BAI. STAI is used for measuring S-Anxiety (intensity of current feelings) and T-Anxiety (frequency of feelings). It can be concluded that nail colors can reduce the intensity of current feelings and frequency of feeling anxiety of participants in both groups. BAI is a measure of anxiety focused on physiological sensation. The participants in both groups had moderate anxiety [12]. The reduction of BAI scores indicated that the anxiety of participants in both groups was reduced after 1-week application of nail polish.

There was no relationship between satisfaction with nail colors and emotional changes after nail polish application in both groups. It can be concluded that the changes in the emotional problems of the participants might be due to the effect of color which is not related to the satisfaction with the color.

Conclusion

This study is based on the theory of color therapy. The participants applied nail polish with different colors based on their emotional problems such as depression,

complicated, lack of confidence, roughness, anxiety, weakness, and confusion. The result showed a significant decrease in emotional problems and a decrease in anxiety levels after nail polish application. It could be concluded that colors have an effect on the emotional and psychological of participants. Therefore, the results from this study might be useful for advertising and marketing nail polish in the future.

Limitation

This study concerning about the emotional data and there were many confounding factors affecting the results. Further studies need to perform the improvement of this work such as selecting the participants that have the same problems, each group having the same number of participants, and prolonging the experimental period to obtain more accurate results.

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A comparative study for clinical efficacy and safety between a combination of fractional radiofrequency and microneedling with fractional radiofrequency alone in the treatment of striae distensae

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ABSTRACT

Introduction: Striae distensae present with structural change of collagen bundles, elastolysis and cellular infiltration, that led to skin flattening, discoloration, and scarring. There is no standard treatment nowadays. Fractional radiofrequency (RF) is a radio transmission that generates thermal effect that promote wound healing effect and stimulate collagen and elastin production. Microneedling is an alternate modality that can also stimulate collagen production.

Objective: This study aimed to compare the efficacy and the safety of a combined fractional radiofrequency and microneedling versus fractional radiofrequency alone for treating striae distensae.

Methods: This was a prospective, randomized-controlled, assessor-blinded, intra-individual split-side comparative, experimental study. Inclusion criteria included all participants aged 18-50 years with Fitzpatrick's skin type III and IV with bilateral sides of striae alba. All participants were randomly assigned to be treated with combined fractional RF and microneedling therapy on one side of the body or fractional RF alone in contralateral side for 3 sessions. The primary outcome was the Global Aesthetic Improvement Scale (GAIS). The secondary outcomes included the width and length of striae, patient's satisfaction's score, and adverse events.

Results: There were 22 participants with the mean (SD) of 29 (7.8) years and 90.9% were female (n=20). The GAIS, assessed by two blinded dermatologists was significantly better improved in the combination group than fractional RF alone group at 4, 8, and 12 weeks ($p < 0.05$). The striae width and length significantly decreased in both groups without significant differences. There was no difference in patient's satisfaction score at week-12 visit between the 2 groups. Common adverse event included post-inflammatory hyperpigmentation (PIH) (36.4%) in the combination group and (27.3%) in fractional RF alone group without significant difference.

Conclusion: A combination of fractional radiofrequency and microneedling had better clinical efficacy in term of GAIS than fractional radiofrequency alone in treating striae distensae.

Keywords: striae distensae; fractional radiofrequency; microneedling

Introduction

Stretch marks or Striae distensae (SD) are common skin problems. It looks like a long line or streak on the skin. It is found in females 2.5 times more than males and is common in pregnant women. The common locations were on the lateral side of the thighs and lower back in adolescent male and on the thighs, upper arms, hips, and chest area in female teenager [1]. Striae distensae can occur from various etiologies, including growth spurts, pregnancy, obesity, collagen disorders

like Marfan syndrome, hormonal abnormalities such as Cushing's syndrome and long-term use of external steroid [2,3]. Striae have two stages. The first stage is characterized by pink, red, or purple streaks and may be accompanied by itching, known as "striae rubra". Later, striae fade into white or the same color as the skin together with the flattening of the skin, which look like skin scarring, known as "striae alba" [3,4]. Treatment of newly occurrence or early stage of striae is highly responsive than older striae [5]. The pathogenesis of

stretch marks is not truly known but hypothesize that to be related to the change in the composition of the extracellular matrix, including fibrillin, elastin, and collagen [6]. Although striae may not cause major impacts but can cause skin problems because of its thinner and impaired skin integrity than usual. It results in itching or blistering more easily. Striae often impact their psychological effects such as anxiety and stress especially in women. There is no specific treatment on striae and most of them disappointingly showed with non-promising result. Previous study showed that laser treatment is more effective than topical medications. However, the efficacy of the treatment is uncertain. Moreover, some lasers have side effects, and the results are not satisfactory [7]. One promising treatment for striae is radiofrequency (RF) wave. RF wave is based on the principle of resistance to tissue current flow in a radio frequency field [8]. RF can be divided into three categories according to the number of electrodes used to generate electricity: monopolar, bipolar, and multipolar. The principle of radiofrequency is different from the laser. It does not involve chromophores absorbing energy, so even dark-skinned people can use it safely [9]. Fractional RF produces thermal effect to small areas of skin, causing the injury in alternating rows with normal tissue, resulting in better wound healing effect [10,11]. Following RF treatment will stimulate the production of collagen and new elastin, a change in the structure of the dermis layer, and make the skin smoother [12]. Fractional RF can be used for the treatment of various dermatological diseases, including wrinkles, and acne scars, with few side effects [10-12], even in darker skin [13]. Microneedling or percutaneous collagen induction therapy has recently been increasingly used in dermatology. It can treat atrophic acne scars, rhytides, surgical scars, skin rejuvenation, and striae [14]. Microneedling is also safe, cost-effective, and can be used to treat a large area. The other advantage of microneedling is that it takes shorter down time for skin recovery period, safe to be used on darker skin [14,15]. This study aimed to determine clinical efficacy and safety of the combination between fractional RF, and skin microneedling in striae distensae by comparing with fractional RF alone.

Methodology

This study is a prospective, randomized-controlled, assessor-blinded, intra-individual split side comparative study. The study protocol was reviewed and approved by Mae Fah Luang Ethics Committee on Human Research with COA number 317/2021.

Inclusion criteria

Participants aged 18 - 50 years with Fitzpatrick's skin type III or IV with bilateral sides of striae alba at buttocks or thighs.

Exclusion criteria

Participants who have had any laser, radiofrequency, chemical peeling, dermabrasion, or microdermabrasion at the study site within 6 months, oral steroid within 3 months, vitamin A derivative within 6 months in oral form and 3 months in topical form prior the study, intralesional collagen, fat (autologous fat transfer), dermal filler or other mesotherapy within 12 months, pregnant women, breast-feeding, concurrent skin lesions at the enrollment visit such as atopic dermatitis, skin infection, or photosensitivity rashes, previous history or current infection of herpes simplex (HSV) or herpes zoster virus (HZV), history of hypertrophic scar and keloid, or abnormal wound healing, current cigarette smoking, overweight or obese if body mass index greater than or equal to 25, cardiac pacemaker or metallic implantation and history of allergic reaction to local anesthetic agents such as lidocaine or prilocaine.

Discontinuation criteria

Participants who developed serious adverse effects from fractional radiofrequency or microneedling; participants who did not comply with study protocol or loss to follow-up visit or pregnancy during the study period or weight gain at least 10% from the baseline during the study period.

All participants require the measurable lesion of striae at both sides of their buttocks or thighs, with total area per side at least 3 but not more than 10 cm². All participants were randomly assigned by block randomization, to receive either a combination of fractional RF and skin microneedling on the one side of the body or fractional RF alone on contralateral side. Photographic examination was performed using high-resolution digital camera (Canon 5D MkIII, Tokyo, Japan) for clinical evaluation by blinded dermatologists. The measurement of the width and length of striae using caliper were performed.

Anesthetic cream (2.5% lidocaine- 2.5% prilocaine) was applied to numb the skin using occlusive dressing. Fractional RF used in this study is the Fractora® handpiece with a 60-pin tip powered by the BodyTite platform (InMode, Yokneam, Israel). The parameter of fractional RF will use the energy with 20-25 mJ per pin to cover all the given striae area without a skip area for two passes. A treatment using microneedling (Dermapen, Dr.pen®, Guangzhou Ekai Electronic Technology, Guangdong, China), uses disposable 42-needle and 3 mm depth was undergone on the assigned combination side after the completion of Fractional RF for 2 passes or reach clinical endpoint with fine pinpoint bleeding. Topical mupirocin ointment will be applied immediately after the procedure. The follow-up visit will be at 4th, 8th, and 12th week after enrollment. The adverse effect was reported.

Primary outcome was global aesthetic improvement scale (GAIS) from photographic examination on each visit, assessed by two blinded dermatologists and given scoring range from (1) to (4)

as follows; (1) = 1-25% improvement, (2) = 26-50% improvement, (3) = 51-75% improvement, (4) = improvement more than 75% from the baseline visit. Secondary outcomes include the width and length of striae on each visit. Overall participant's satisfaction score using a 5-score rating scale as follows; (0) = unsatisfied, (1) = less satisfied, (2) = moderately satisfied, (3) = very satisfied, (4) = most Satisfied. Adverse effects were reported.

Statistical analysis

The sample size was calculated by using the formula for two samples, comparing two proportions in the dependent outcome, based on the results of a study from Naeni et al. (2016) [49] with 80% power ($\beta=0.20$) and type I error of 5% ($\alpha = 0.05$) with a total of twenty-five subject enrollment plan. Descriptive statistics were reported with the means and standard deviation or frequency and percentage. Two-way, repeated measure Analysis of variance (ANOVA) was used to compare continuous data between the two groups at different visits. For comparing two categorical data, such as GAIS score greater than or equal to two points and adverse effect, the McNemar test was used. *P* value less than 0.05 was clinical significance. All data were analyzed by using Statistical Package for the Social Sciences (SPSS) for window version 23.0 (SPSS, Chicago, IL, USA).

Results

This study was conducted between January 18 to May 29, 2022. A total of 25 volunteers were initially screened and enrolled, but 3 subjects dropped out because of COVID-19 situation effect. AS total of 22 subjects completed the study protocol. The mean \pm SD age was 29 ± 7.8 years. Twenty participants (90.1%) were female. There were 13 participants (59.1%) had Fitzpatrick's skin type III, and nine participants (40.9%) had type IV. The locations of striae were at buttock (63.6%) and thigh (36.4%) (table 1).

Primary outcome

Using the McNemar test to compare the proportion of at least score (2) or more than or equal to 26% improvement by compare with eh baseline, the combination of fractional RF and skin microneedling had significantly better in term of GAIS measurement than fractional RF alone group at 4, 8, and 12 weeks ($p = 0.0390, 0.0010, \text{ and } 0.0310$, respectively) (table 2).

Secondary outcome

The mean width at baseline visit in the combination treatment group was 0.31 ± 0.07 cm, while the mean width of the fractional RF group was 0.31 ± 0.07 cm. When comparing the widths of the two treatment groups at baseline using paired *t* test was found with no statistically significant difference ($p\text{-value} = 0.8759$). Both treatment groups were statistically reduced the striae width when comparing with the baseline ($p\text{-value} < 0.001$). Moreover, there were no significant difference between the groups at different visits ($p\text{-value} > 0.05$) (figure 1). The length of striae follows the same trend as the width. The mean pre-treatment length in the combination treatment group was 8.1 ± 3.1 cm and was 8.09 ± 2.5 cm in the Fractional RF alone group. After treatment, the length in each group was significantly reduced compared to the baseline ($p\text{-value} < 0.001$). Nevertheless, when comparing between the two group of treatments, found no statistical difference. A combination of fractional RF and microneedling group found 68.3% most satisfied (score = 4) or very satisfied (score = 3) satisfaction score and 45.5% in fractional RF alone group with no significance ($p\text{-value} = 0.6871$). There were 8 participants (36.4%) were in the combination treatment group and 6 participants (27.3%) in the Fractional RF alone group, developed post-inflammatory hyperpigmentation without significant difference ($p\text{-value} = 1.00$).

Table 1 Base characteristics

Clinical data	Results (n = 22)
Age	
mean (years) \pm SD	29 ± 7.8
Age < 30 yr., n (%)	10 (45.5)
Age \geq 30 yr., n (%)	12 (54.5)
Gender, n (%)	
Male	2 (9.1)
Female	20 (90.1)
Fitzpatrick's skin type, n (%)	
III	13 (59.1)
IV	9 (40.9)
Location, n (%)	
buttock	14 (63.6)
thigh	8 (36.4)

Table 2 Global aesthetic improvement scale (GAIS) comparing two groups' results at each visit.

GAIS, n (%)	Fractional RF + Microneedling group (n = 22)	Fractional RF group (n = 22)	p-value*
Week 4			
(1) = improve 1 - 25%	8(36.4)	15(68.2)	0.0390
(2) = improve 26 - 50%	13(59.1)	6(27.3)	
(3) = improve 51 - 75%	1(4.5)	1(4.5)	
(4) = improve > 75%	0	0	
Week 8			
(1) = improve 1 - 25%	6(27.3)	17(77.3)	0.0010
(2) = improve 26 - 50%	7(31.8)	5(22.7)	
(3) = improve 51 - 75%	7(31.8)	0	
(4) = improve > 75%	2(9.1)	0	
Week 12			
(1) = improve 1 - 25%	4(18.2)	10(45.5)	0.0310
(2) = improve 26 - 50%	7(31.8)	9(40.9)	
(3) = improve 51 - 75%	9(40.9)	3(13.6)	
(4) = improve > 75%	2(9.1)	0	

*McNemar test comparing GAIS of greater or equal to two scores (improvement $\geq 26\%$) between two groups.

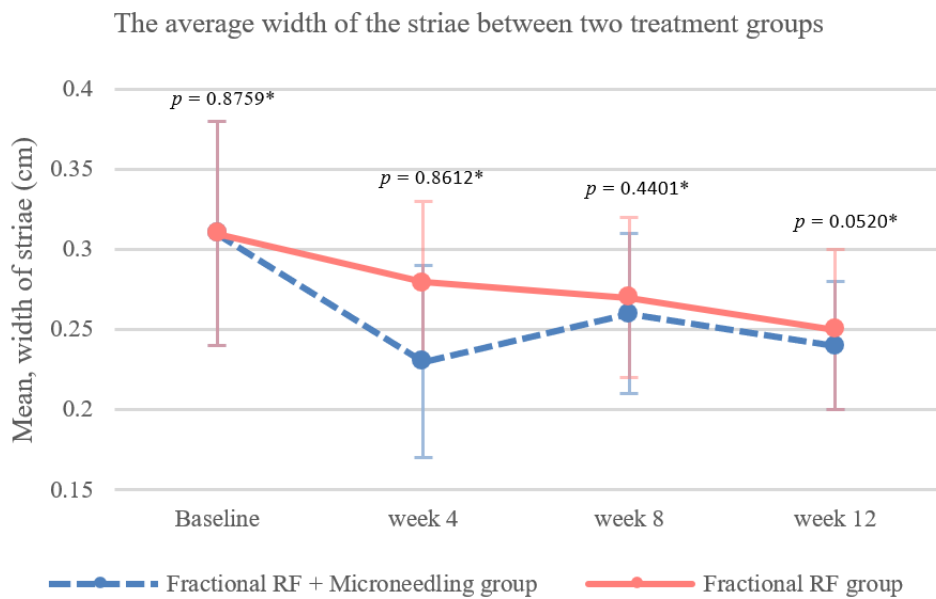


Figure 1 A comparison of striae width at different visits between the two groups

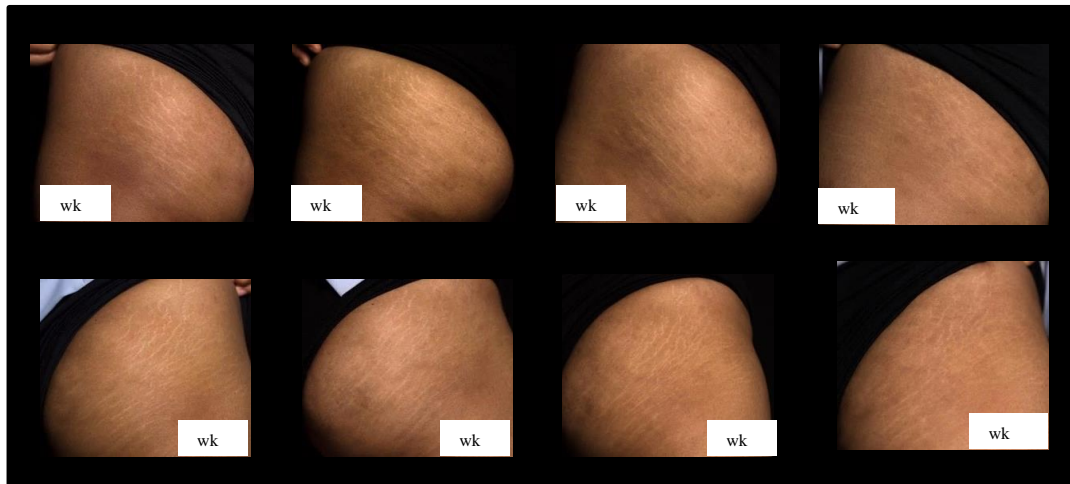


Figure 2 Representative clinical photographs for evaluation in GAIS in each visit compared to baseline. The upper row shows the striae in a combination of fractional RF with the microneedling group. In comparison, the lower row shows the striae in the fractional RF alone group. By week 12, the striae from the upper image have a faded appearance. The skin is smoother than in the picture below.

Discussion

This research is an early study comparing the combination of fractional RF and microneedling treatment to the fractional RF treatment alone. The primary outcome is the overall improvement of striae from two dermatologists' scores using GAIS. The combination treatment has a better score than the fractional RF alone, with statistically significant differences in the 4th, 8th, and 12th week (p -value < 0.005). This result is consistent with research by Seong et al (2021) [17] and Ryu et al (2013) [18], which found that fractional radiofrequency microneedle was effective in treating striae based on overall clinical evaluation by dermatologists as well. There was a statistically significant reduction in width and length in both combination therapy and fractional RF alone group (p -value < 0.001). Consistent with the research by Phongsrihadulchai et al (2016) [19]. They found that the width and length of striae were statistically significantly reduced when measured by caliper. However, when comparing treatment between the two groups, there were no statistically significant differences. The main treatment side effects were post-treatment hyperpigmentation (PIH), with an incidence of 36.4% in combination treatment and 27.3% in fractional RF alone. This incidence of PIH was higher than the reported PIH from Ryu et al. (2013) [18], which happened with 20% PIH from non-ablative fractional microneedle RF and 18.1% PIH from nano fractional radiofrequency in Phongsrihadulchai et al study (2016) [19]. This figure is close to research by Yang YJ et al (2011) [20] using a fractional Erbium glass laser reporting 36.4% PIH, compared to a fractional CO₂ laser producing 81.8% PIH. There was no statistically significant difference in participants' satisfaction between the two groups. However, in fractional RF

alone treatment, one participant was dissatisfied with the treatment because she discovered that there were more striae than before. When taking more history, she said she had gained 2 kg between week 8 and week 12. The limitation of this study is that there was a relatively small number of volunteers and more lost to follow-up of participants than expected dropout calculation due to the COVID-19 situation. So, the suggestion for further study may weight participants in each visit as weight changes may affect the research results. Should increase the time for follow-up, such as follow-up after the last treatment for three months. to see the long-term treatment effect. Furthermore, it may add the depth of striae to the width and length measurement using a 3D camera such as Antera 3D™.

Conclusion

A combination of fractional radiofrequency and microneedling had better clinical efficacy in term of GAIS than fractional radiofrequency alone in treating striae distensae.

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The Protective Effects of Marine Microalgae Extract on Skin Cells-induced Sun Ray

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ABSTRACT

Introduction: In the present study, two kinds of marine microalgae, *Tisochrysis lutea* (Coccolithophyceae) is a single microalga widely used as live food in aquaculture. *Tetraspora gelatinosa* (Tetrasporaceae) is spherical to elliptical cell, arranged two-by-two or four-by-four, with 2 pseudoflagella extending beyond the mucilage. They are able to produce high value chemicals which can be applied in feed and food, pharmaceutical, nutraceutical and cosmeceutical industries.

Objective: To determine effects of *T.lutea* and *T. gelatinosa* extracts on skin cells functions.

Methods: Dried microalgal biomass of microalgae were extract with ethanol. The biological activity of microalgae extracts was investigated on skin cells such as keratinocyte cells (HaCaT) and melanoma cells (B16F10). The protective effect against photo-induced inflammation, oxidative stress and melanogenesis were investigated. Cytotoxicity of microalgae extracts were determined using WST-assay. Sun ray was treatment to HaCaT cells for inflammation and oxidative stress effect. and B16F10 cells was treated sun ray to inducing and melanin production.

Results: The content of the biochemical components in two marine microalgae showed total carbohydrate and protein content were the main component of *T. gelatinosa*. The effect of *T. lutea* and *T. gelatinosa* were exhibited to suppressing cytokine secretion, melanin content, and ROS production on sun ray-induced skin cells. Moreover, *T. gelatinosa* was showed significant protective effect on ROS scavenging.

Conclusion: *T. lutea* and *T. gelatinosa* are marine microalgae that have benefit on skin cells to protect sun ray induce cell damage and skin pigmentation. The protection activity on keratinocyte cells including anti-inflammation via cytokine PGE₂ suppressing and anti-oxidative by ROS scavenging from sun-ray stimulation. Therefore, both of marine microalgae may have the potential to use as an active ingredient in cosmetic or cosmeceutical products.

Keywords: marine microalgae; sun-ray; anti-inflammation; ROS scavenging; melanin content

Introduction

Currently, marine microalgae have been attracting attention as resources for new metabolites and biotechnologies. They are predominately aimed at applications with high added value given that algal biomass is composed of proteins, polysaccharides, essential fatty acids, pigments, vitamins, and minerals, all of which are of considerable interest in the preparation of natural products or bioactive compounds

[1]. They can defend its cellular components by the counter production of primary metabolites such as chlorophyll, phycocyanin, palmitic acids, oleic acids, vitamin and etc [2]. Secondary metabolites are also generated under stress environment condition and accordingly produce various high-value metabolites such as antibiotic and antimicrobial agents, for example, working against inflammation and tumor, or with anti-viral and immunomodulating actions [3,4]. There is a

growing worldwide interest toward finding new, safe and powerful bioactive compounds from microalgae. Those metabolite productions can be applied in food, pharmaceutical, nutraceutical, cosmeceutical which can be applied to repair damaged skin, prevent blemishes, and inhibit the inflammatory process. Additionally, the various bioactive agents of microalgal extract could help to accelerate the healing process and maintain skin moisture [5,6].

The diverse pharmacological activities can be found in different species of microalgae, a specific marine microalgae species can be used as cosmetic applications from many different bioactive compounds. For instance, *Chlorella* is a good candidate for polysaccharide to help moisturize and thickener agent [7] and for chlorophyll to mask odors in dentifrices and deodorants [8]. *Dunaliella salina* can be used to produce carotenoids to support antiglycation and anti-inflammatory [9]. *Spirulina* can accumulate a large amount of phycocyanobilin and phycoerythrobilin to use as antioxidant for sunscreen formulation on health of the dermis and the skin elasticity, reduction of skin hyperpigmentation, protection against photoaging and inhibition of reactive oxygen species: ROS-induced damage to the dermis [10,11]. *Tisochrysis lutea* (also named as *Isochrysis galbana* T-Iso) [12] is widely used in aquaculture due to its high content in polyunsaturated fatty acids, particularly in docosahexaenoic acid (DHA). Additionally, this microalga is applied in cosmetics, particularly in skin photoaging protection and antiaging because of its high fucoxanthin content [13,14].

The sun ray comprises a various range of electromagnetic radiation [15], including ultraviolet (UV, approximate range of wavelength from 180 to 380 nm), visible (Vis, approximately from 380 to 800 nm), and infrared light (range 1–3 μ m approximately). The sun's ultraviolet light can cause major damage to the skin cells. Too much exposure to UVB rays can lead to sunburn. UVA rays can travel more deeply into the skin than UVB rays, but both can affect your skin's health. UV light can lead to pathological UV-induced ROS production with affecting the enzyme catalase and up-regulating nitric oxide synthase (NOS) synthesis. It may also cause a decrease in protein kinase C (PKC) expression leading to increased ROS production [16]. Exposure the UV radiation triggers the release of prostaglandins (PGs), which is produced abundantly by keratinocytes in UV-exposed skin. This is the major and most effective metabolite generated by COX-2 activity and is considered to be a potent mediator of inflammatory responses. These data suggest that PGE2 plays a key role in UV radiation-induced immunosuppression [17]. UV radiation exposure is the stimulant for melanin synthesis. Enzyme tyrosinase is the key factor in melanogenesis, which catalyses tyrosine to L-DOPA and oxidation of this o-diphenol to dopaquinone. The oxidation of DOPA-quinone by

cyclisation produces cyclo-DOPA and DOPA-chrome. Dopachrome continues the route for the formation of dark/brown eumelanin [18]. In this study, we investigated the effect of marine microalgae extracts from two marine microalgae on valuable application of cosmetics. The effects of *T.lutea* and *T. gelatinosa* extracts were determined on skin cell-induced sun ray. The anti-inflammatory and anti-oxidative effects were observed on keratinocyte (HaCaT) cells. While the anti-melanin production was determined on melanoma (B16F10) cells

Methodology

Microorganism

Two marine microalgae were obtained from the algal excellent center of Thailand institute of scientific and technological research (TISTR). *Tisochrysis lutea* TISTR 11470 (Haptophyta, Coccolithophyceae) and *Tetraspora gelatinosa* TISTR 11440 was collected from Mu Ko Chumphon National Park, Chumphon province and *Tetraspora gelatinosa* TISTR 11440 (Chlorophyta, Tetrasporaceae) was obtained from Cha-Am beach, Phetchaburi province. Those were located in the Gulf of Thailand.

Algal extraction

At the 10th day of cultivation, algal extraction was performed according to Maadane et al. [6] with some modifications. Dried microalgal biomass was ground well using a sterile mortar and pestle. 50 g of the dry cell was mixed with 100 ml of ethanol and placed at room temperature for 2 hours under dark. Cells were disrupted by a mechanical homogenizer (Daihan HG-15A, Korea) at a speed of 8,000 rpm for 10 minutes in an ice-bath at the controlled temperature of -5°C to avoid overheating. Samples were centrifuged at 6,000 rpm for 5 minutes and then the pellet was repeated extract. The pooled supernatant was evaporated to dryness at 40°C and the residue was kept at -20°C before analysis. The extracts were analysis chemical component: protein [19], carbohydrate [20] flavonoid [21], and phenolic content [6]. The bioactivity assay was analysis on skin cells including keratinocyte and melanocyte cell line.

Cell culture

Human keratinocyte cell line (HaCaT; CLS cell lines, Germany) and mouse skin melanoma cell (B16F10; ATCC® CRL-6475) were grown and maintained in Dulbecco's modified eagle medium (DMEM) (GIBCO, USA) containing 10% (v/v) fetal bovine serum (GIBCO, USA) and 1% of Antibiotic/Antimycotic Solution (GIBCO, USA) in humidified atmosphere incubator with 5% CO₂ at 37°C. Cells were seed in 96 well plate at density of 5x10⁴ cells/well for 24 hours and incubated with various concentration of algae extract. Cells were treated with sun ray (UVA+B+IR) for 22 second before bioactivity analysis.

Cytotoxicity assay

Cytotoxicity was determined using WST-1 assay (Bio Vision, Milpitas, CA, USA). Cells were treated with various concentrations of extracts for 24 hours. Then, 100 µl of WST-1 solution was added and incubated for 30 minutes before measuring the absorbance at 450 nm.

Evaluation of photo-induced cytokine production

After sun ray-induced and 48 hours post-incubation, the supernatant of HaCaT cells were collected for cytokine assay. Prostaglandin E2 (PGE₂) were quantified via the enzyme-linked immunosorbent assay (ELISA) kit (Cayman, USA) according to the manufacturer's instruction and modified from Prasad et al [22].

Evaluation of photo-induced ROS production

The ROS scavenging activity was performed according to Masaki et al. [23] with modifications. HaCaT cells were induced oxidative stress with sun ray and post-incubate for 5 minutes and added the ROS fluorescent dye solution (ROS detection kit, Abcam, USA). Cells were incubated in a 5% CO₂ incubator at 37°C for 45 minutes. Cellular ROS production were quantified the intensity of fluorescent using In Cell Analyzer (InCell Pro2000, GE Healthcare, UK).

Evaluation of photo-induced melanin production

B16F10 cells were stimulated by sun ray and post-incubation for 48 hours. Cells were washed by PBS before added 100 µl of 2 M NaOH solution. Cell was incubated in a 5% CO₂ incubator at 60°C for 2 hours. The absorbance was measured at 405 nm following to Zhou et al. [24] with modification.

Results

Chemical compounds

The biochemical contents in two marine microalgae (figure 1) are shown in table 1. Total carbohydrate and protein contents were the main component of *T. gelatinosa* approximately 217.45±3.34 and 145.90±1.75 mg/g respectively. On the contrary, phenolic and flavonoid levels were higher in *T. lutea* compared with *T. gelatinosa*.

Table 1 Components of marine microalgae.

Components	<i>T. lutea</i>	<i>T. gelatinosa</i>
Total protein (mg/g)	8.99±0.63	145.90±1.75
Total carbohydrate (mg/g)	12.84±0.14	217.45±3.34
Phenolic (mg GAE/g)	5.36±0.39	0.09±0.02
Flavonoid (mg CE/g)	13.73±0.30	4.10±0.64

Results are expressed as mean ± SD (n = 3). Gallic acid equivalent (GAE) and catechin equivalent (CE).

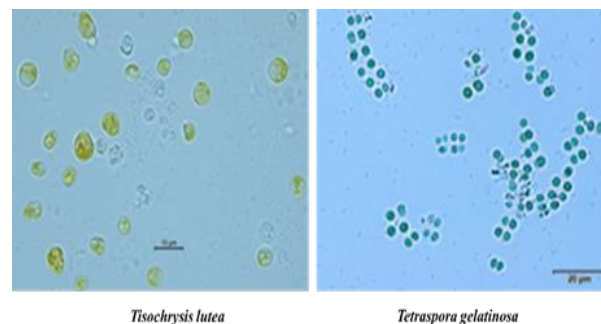


Figure 1 Morphology of marine microalgae.

Cytotoxicity on skin cells

Cytotoxicity of *T. lutea* and *T. gelatinosa* was measured cell viability using WST-1 assay. *T. lutea* and *T. gelatinosa* extracts did not show cytotoxicity to skin cells up to concentration of 0.50 mg/mL (data not shown).

Inhibitory effect on photo-induced cytokine production

The inhibitory effect of algae extract on cytokine (PGE₂) production was measured using ELISA assay in sun ray-stimulated HaCaT cells. Sun ray-induced cells was increasing PGE₂ level more over than control cell. The protective effect of *T. lutea* and *T. gelatinosa* extracts at concentration of 0.125-0.5 mg/mL were shown to decreasing PGE₂ level from sun ray induction more than 30%. The inhibition ratio of *T. lutea* and *T. gelatinosa* on cell-induced inflammation was not significant difference (figure 2).

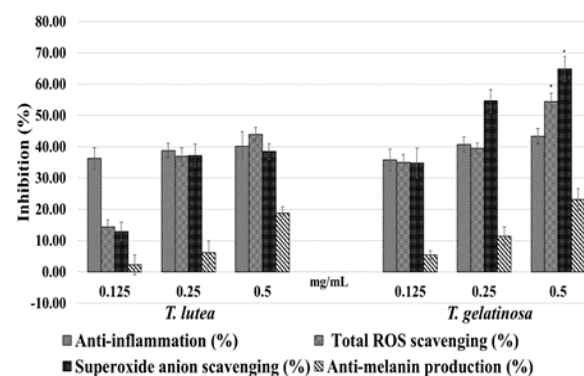


Figure 2 Effects of algae extracts on photo-induced skin cells. Values are expressed as mean±SD. Statistical significance was evaluated using the turkey test. $p < 0.05$ compared between group.

Inhibitory effect on photo-induced cellular ROS production

To determine the effect of algae extracts on anti-oxidative in HaCat cells was performed with cellular ROS scavenging activity. Total ROS (green) and superoxide anion (red) was detected with fluorescence intensity. HaCat cells induced with sun ray showed increasing of total ROS and superoxide anion

production (Figure 3). The result showed that *T. lutea* and *T. gelatinosa* were suppressing the intensity of ROS production after sun ray induction. In additional, radical scavenging effect of *T. gelatinosa* extracts at 0.5 mg/mL was significantly greater than *T. lutea* (figure 2).

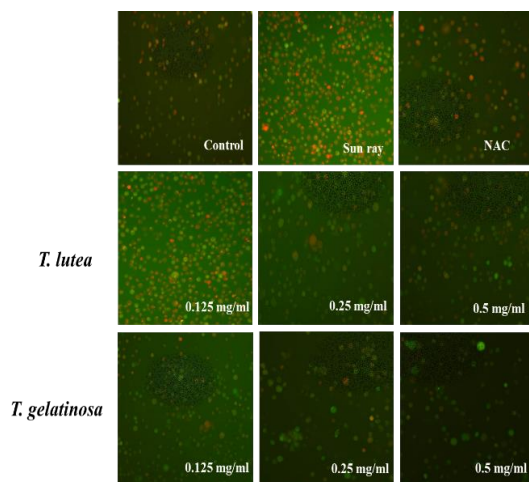


Figure 3 Effects of microalgae on sun ray induced oxidative stress in keratinocyte (HaCaT) cells, stained with immunofluorescence. Fluorescence images were merged, in the presence of total ROS (green) and superoxide anion (red).

Inhibitory effect on photo-induced melanin production

To investigate the anti-melanogenic effect of algae extract, cell was treated with sun ray and analyzed for melanin content. The result examined *T. lutea* and *T. gelatinosa* were decreasing melanin content after stimulated with sun ray in dose dependent manner as show in figure 4. The result showed that the inhibition effect on B16F10 cells melanin secretion of *T. lutea* slightly higher than *T. gelatinosa* extracts but not significant difference (figure 2).

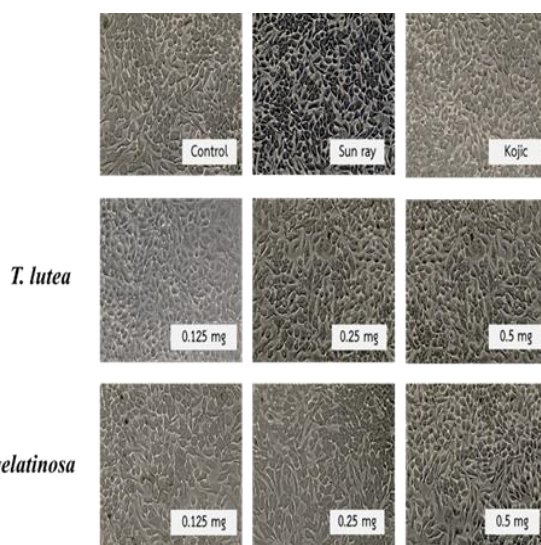


Figure 4 Effects of microalgae on sun ray induced melanin production in melanoma (B16F10) cells.

Discussion

The beneficial effects of marine algal extracts showed the various biological activities against skin disorders including wrinkles, skin inflammation, hyperpigmentation [25,26,27] The content of the main biochemical components in microalgal cells varies depending on the investigated microalga species [28,29]. This study showed that *T. gelatinosa* gave the highest total protein and carbohydrate. This reported also similar the total carbohydrates in the four marine microalgae varied from 54 to 235 mg g⁻¹ DW [30] and total protein content of marine microalgae *Chlorella* was 45 percent of total organic matter [31]. The high total carbohydrate of *T. gelatinosa* may relate with polysaccharide or sulphate polysaccharide that was found in marine microalgae. Polysaccharide from most marine microalgae are heteropolymers, constituted mainly of xylose, galactose, and glucose in different proportions [32].

The significant anti-oxidant activity of *T. gelatinosa* which contain highest carbohydrate was supported in numerous reported. Dvir et al. [33] claimed that sulphate polysaccharides released by marine microalgae may not only function as dietary fibre, but have also illustrated the ability to prevent the accumulation and the activity of free radicals and reactive chemical species. According to Park et al. [34] demonstrated that sulphate polysaccharide from green microalgae *Haematococcus lacustris* had anti-inflammatory activity. Moreover, bioactive peptide and proteins from *Chlorella* exhibited anti-inflammatory activity [35,36] and the possibility of the glycoprotein from the red marine microalga *Porphyridium* sp. contributed the antioxidant properties [37]. The polysaccharides isolated from red green and brown

marine macroalgae/seaweed possess antioxidant potential [38]. Sun et al. [39] found that polysaccharides degradation from algae *Pavlova viridis* and *Sarcinochrysis marina* Geitler et al. showed antioxidant activity in DPPH radical and hydroxyl free radical scavenging. Luo et al. [40] found that *Spirulina platensis* polysaccharide has a strong antioxidant activity and could be used as an antioxidant in Sausage to extend its shelf life. However, although diverse biological activities of marine carbohydrates have been determined, their detailed molecular mechanisms and target proteins are not fully understood [41]. The results of *T. lutea* and *T. gelatinosa* were showed inhibition effect on melanin content in B16F10 cells similar with fresh water green algae (*Prasiola japonica*). *P. japonica* ethanol extract (Pj-EE) was suppressed the transcription of genes encoding matrix metalloproteinases (MMPS), which were induced in HaCaT cells by hydrogen peroxide (H₂O₂) treatment and reduced the melanin secretion and content in B16F10 cells [42]. Study from Wu and co-workers found that C-PC from *Spirulina* sp., was inhibits melanin biosynthesis in B16F10 murine melanoma cells [43]. A novel peptide isolated from *Pavlova lutheri* demonstrated inhibitory properties against α -Melanocyte Stimulating Hormone-induced melanogenesis via melanin content, tyrosinase inhibition in B16F10 melanoma cells, and also decreased melanogenesis-related proteins [44]. However, the color and the active ingredients of the algae should be related to the protective effect from photo induction such as the green color, phenolic and flavonoid. The sun protection factor (SPF) should be further determined to clarified the mechanism of action. Moreover, in view of the industrial scale culture of microalgae have the potential to become antioxidants in foods and cosmetics.

Conclusion

T. lutea and *T. gelatinosa* are marine microalgae that have benefit on skin cells to protected sun ray induce cell damage. Melanin content from sun ray induction on melanoma cells was decreasing from protective effect of both marine microalgae. The protection activity on keratinocyte cells including anti-inflammation via cytokine PGE₂ suppressing and anti-oxidative by ROS scavenging from sun-ray stimulation. While, *T. gelatinosa* showed stronger effect of ROS scavenging activity than *T. lutea*. However, both of marine microalgae may have the potential to use as an active ingredient in cosmetic or cosmeceutical products.

Competing Interests

The authors declare that they have no competing interests

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Author contributions

TM participated in research design, discussion of result and final revision. NC prepared the sample extract and also aided in the discussion of result and manuscript drafting. IP contributed to research design, performed the experiment, and analyzed the result.

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Development of Problem-Solving Skill by Project-Based Learning in Physical Therapy Students, Mae Fah Luang University

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ABSTRACT

Introduction: Project-based learning is a classroom approach that based on the problems or questions that allows student to work together on a project. The engagement of the student in the investigation of problems could help student to develop problem - solving skill which could be applied in physical therapy in sports course. Therefore, this study hypothesized that the use the project-based approach in physical therapy in sports course could improve problem-solving skill in physical therapy student.

Objective: To evaluated the effect of project-based learning on problem-solving skill in physical therapy in sport course.

Methods: Students worked on the athletes to identify the problem and then did the searching and concluded the all of the data for planning and constructed the project. The problem-solving skill will be evaluated on the first and final week. The satisfaction survey of this course was taken by the students after the project by the university system.

Results: The problem-solving skill is significantly improved by project-based learning in physical therapy in sports course and the satisfaction after this course was in high satisfaction level.

Conclusion: The project-based learning in physical therapy in sports improved the problem-solving skill of the physical therapy students in this course.

Keywords: *problem-solving skill, project-based learning, physical therapy in sports*

Introduction

Project-based learning is the learning through the project. The projects-based learning is consisted of complex tasks. It is involved the demanding the questions or problems that give the students a chance to develop the problem-solving, decision making and allow students to work together to work continuously through the project and present the work in the classroom (1–3). The student will find the solutions for the questions or problems that cause the problem-solving process especially in the process of the project-based learning together in a group that requires lots of skills more than problem-based learning.

The physical therapy in sport course includes the physical therapy management in athlete pre, during, and post even; assessing, diagnosing sport injury; physical

therapy and rehabilitation; promoting physical fitness and preventing sport injury; adhesive taping, providing the knowledge regarding exercise, principles of nutrition for athlete training; contraindications and danger stimulants and addictive substance using; procedure of transferring the injured athletes and sport for the handicapped. Moreover, this course is focused on experience learning. Therefore, the project-based learning is one of the learning procedures which could facilitate student in experiencing learning, working together as a team, develop the ability of problem-solving skill. Furthermore, the previous studies showed that the project-based learning demonstrated some development in self-directed learning skill and also 21st century skill that include the problem-solving skill (4,5) due to the student could create knowledge by themselves

by designing planning performing on a project that facilitate the problem-solving skill. Therefore, for promoting the problem solving-skill, this study hypothesized that project-based learning in physical therapy course could improve problem-solving skill in physical therapy student.

Methodology

This study recruited the physical therapy student 4th year who enrolled in physical therapy in sport course year 2019 and 2020.

Outcomes measurement

Problem-solving measurement by questionnaire that developed by the content of bloom's taxonomy. The questionnaire has strong agreement from 3 lecturers.

The questionnaire was in rubric scores that had four items totally 16 points. It is consisted of the ability to state the problems of the athlete, the ability to create exercise /program and find the reference following the athlete's problems by thinking critically and the ability to analyze, synthesis and evaluate of the relevant information to solve the athlete problems, the ability to perform exercise/program following the athlete's problems. The scores criteria have 4 scores including excellent (4) which is the ability to do the task 90-100%, superior (3) which is the ability to do the task 70-89%, satisfaction (2) which is the ability to do the task 50-69%, need improvement (1) which is the ability to do the task less than 50%.

Procedures

Student set into 4 groups in year 2019 and 5 group in 2020 the problem-solving skill evaluated by questionnaire first week and final week of the project. The student had worked in a project for 8 weeks. There were 5 activities throughout the project which were define the athlete's problems, Data collection and finding the related topic following the problems, project planning , project construction, project presentation.

Statistical analysis

Descriptive statistics showed the score for the first and final week. The Wilcoxon signed-rank test was used for testing the difference between first and final week. The p-value set at ≤ 0.05 .

Results

The result of the activities shown in table 1.

Table 1 demonstrated the learning procedure through the project-based learning

Activities	Result
1. Define the athlete's problem	Student were learning from real situation and define the athlete's problem
2. Data collection and research the related topic following the problem	Student shared knowledge and discussed with group member and lecturer
3. Project planning	Student planned activities and project together
4. Project construction	Student constructed the project and submitted work through google classroom
5. Project presentation	Student presented the project to the class

The students who enrolled in physical therapy in sport course in year 2019 (N = 54) had the score of problem-solving skill of 9.07 ± 1.24 (mean \pm SD) for the first week and 14.00 ± 1.01 for the week.

The data were not distributed normally. The Wilcoxon signed-rank test was used to evaluated the difference between first and final week of project-based learning. The result in Figure 1 showed the significantly improvement of problem-solving after project-based learning ($p = 0.00$).

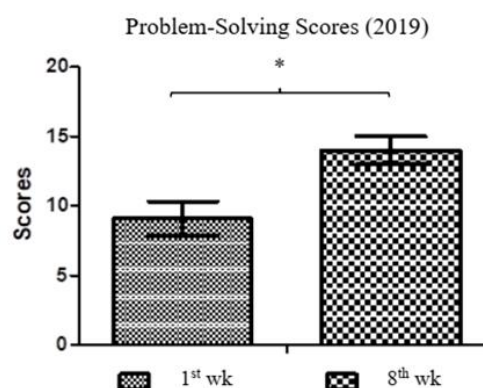


Figure 1 showed the difference between the scores of problem-solving skills in first week and final week

* Show statistically significant difference ($p \leq 0.05$)

The students who enrolled in physical therapy in sport course in year 2020 (N = 50) had the score of

problem-solving skill of 13.90 ± 0.92 (mean \pm SD) for the first week and 14.60 ± 1.21 for the final week.

The data were not distributed normally. The Wilcoxon signed-rank test was used to evaluate the difference between first and final week of project-based learning. The result in Figure 2 showed the significantly improvement of problem-solving skill after project-based learning ($p = 0.00$).

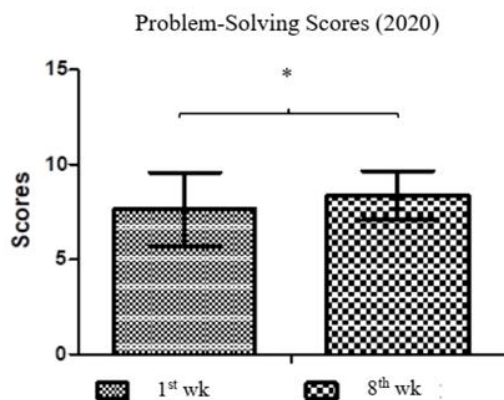


Figure 2 showed the difference between the scores of problem-solving skills in first and final week
* Show statistically significant difference ($p \leq 0.05$)

Satisfaction scores of physical therapy in sport course from the student showed high satisfaction level at 4.8/5 in 2019 and 4.7/5 in 2020.

Discussion

This study demonstrated that the project-based learning significantly improved the problem-solving skill through the process of project-based learning which facilitate the student to carefully indicated the problem and searching all of the solution and systematically working in the activities in a group by their own interests (1,6–9). In order to complete the activities, the student developed the interpersonal skill, computer skill, problem-solving skill, physical therapy skill. Therefore, the student could create the solution for athletes according to their problems which was similar to the previous study that showed the improvement in problem-solving skill in nursing student (10). Moreover, the project-based learning helped students to integrated the knowledge and improve the skill in main competences in engineer students (11). From the learning process of the project-based learning, it could one of the factors that affected the motivation in the student (12).

The project-based learning took a long time to completed the activities. The teaching plan and the amount of other homework in the course should not be too much for the students, otherwise the students could not received the benefit from the project-based learning. The satisfaction level could reflect the appropriate

amount of the work and time from the student which was high satisfaction level in this study.

The project-based learning could be applied to other physical therapy courses which focused on the problem-solving skill, interpersonal skill, computer skill, physical therapy skill. However, this learning style take a lot of time to do the project, the project and the time should be considered appropriately.

Conclusion

The project-based learning in physical therapy in sports improved the problem-solving skill of the students in this course.

Limitations of this study

This study did not provide the data of validity and reliability of the questionnaires. It was the agreement of the lecturer based on the bloom's taxonomy. Therefore, the further study should investigate the validity and reliability of the questionnaires.

Competing Interests

The authors report no declarations of interest.

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Author contributions

Author 1 conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis and wrote the paper.

Author 2 conceived and designed the analysis, contributed data or analysis tools, performed the analysis.

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Ultrasonic Assisted Extraction Enhanced Total Phenolic and Antioxidant Activities from *Aegle marmelos* (L.) Corr. Extract

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ABSTRACT

Introduction: The ultrasonic assisted extraction (UAE) is a green technology to extract bioactive compounds from natural sources. The extraction process showed the high efficiency in part of activity and reducing time. *Aegle marmelos* (L.) Corr. or bael has been used as traditional medicine to dysentery, fever, diabetes, asthma, heart problems. There are various kinds of secondary metabolites have been reports and their pharmaceutical activities.

Objective: To determine total phenolic content and antioxidant activities of two methods of extraction; maceration and ultrasonic assisted extraction.

Methods: The powder of *A. marmelos* was extract by maceration and UAE using water and EtOH as solvents. The extract was investigated total phenolic content and antioxidant activity including DPPH and FRAP assays.

Results: The ultrasonic assisted extraction of ethanolic extract at 15 minutes showed the highest values of total phenolic content at 79.75±0.05 mg GAE/g crude extract. Moreover, the antioxidant activity of extract using UAE at 30 minutes exhibited the most percent inhibition about 39.50% in DPPH assay whereas hot water extraction found the better antioxidant activity in FRAP assay with the value of 355.75 µM/g crude extract.

Conclusion: UAE method was high efficiency in the extraction especially in term of extraction time and bioactivity.

Keywords: *ultrasonic assisted extraction; aegle marmelos; total phenolic content; antioxidant activity*

Introduction

Aegle marmelos (L.) Corr. belongs to Rutaceae family having common name as bael. This plant distributed in the tropical area especially India and Southeast Asia [1]. A part of bael also used as traditional medicine such as dysentery, fever, diabetes, asthma, heart problems, ophthalmia, haemorrhoids and urinary problems [2]. Furthermore, phytochemical constituents of *A. marmelos* were isolated the secondary metabolites including alkaloids, coumarins, flavonoids, phenylpropanoids, tannins and terpenoids [3]. This plant has been reported in various biological activities, for example, anticancer, antidiabetic, antifungal, antipyretic, antioxidant and wound-healing activities. Some of leaves extract showed insulin-like-

hypoglycemic activity [1]. Essential oils of bael exhibited interesting antifungal activity [2]. Maceration, the conventional extraction method. The powdered materials are put in container then filled solvent until covered materials. The container was closed and should have shaken time and kept for 3 days for completely extraction. The extraction depends on polarity of solvent and times. The extract was filtrate to remove marc and the solution was evaporated to obtain extract. This method is suitable for plant material required long exposure. Furthermore, maceration is convenient and safe for thermolabile plants [4]. Ultrasonic assisted extraction (UAE) is a green technology to extract bioactive natural products. The method used the ultrasonic wave properties to break cell wall and then

compounds were extracted. There are two types of ultrasonic extraction, ultrasonic bath and probe ultrasonic equipment. First, ultrasonic bath type is commonly known in the experiment using stainless still bath with ultrasonic transducers. This type of extraction is controlled frequency around 40 kHz and also temperature of extraction process. In contrast, probe type is directly delivered ultrasonic wave to extraction media and operated with frequency at 20 kHz and extract in the reactor [5]. There are some applications of UAE have been published. Ultrasonic extraction of *Laurus nobilis* L. showed total phenolic content about 17.32 ± 1.52 mg/g plant and exhibited antioxidant activity [6]. Moreover, the leaves of *Moringa oleifera* were extracted by UAE that obtained total phenolic content at 328.87 mg GAE/mg and antioxidant activity about 72.44% [7]. Herein, we studied total phenolic content and antioxidant activity of *A. marmelos* extract using ultrasonic assisted extraction compared with conventional extraction.

Methodology

Chemicals and Plant materials

The fruits of *A. marmelos* were collected from Saraburi province, Thailand in 2021. The taxonomy of this plant was identified and has been deposited at Bangkok Herbarium, Bangkok, Thailand (specimen no. Sinee01). The ultrasonic extraction ultrasonic probe and 10 mm diameter of hon. 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from TCI, Japan. Folin-Ciocalteu reagent were obtained from Merck, Germany. Gallic acid and L-ascorbic acid as standard compounds were Sigma-Aldrich. Ethanol for extraction was AR grade.

Sample preparation

The fruits of *A. marmelos* were cut to small pieces then dried in the oven at 50°C for 2 days. The air-dried fruits were blended to powder. The fruit powder was kept in zip lock bag and stored before extraction.

Extraction of *A. marmelos* using maceration and UAE

Maceration, dried fruits of *A. marmelos* were extraction with EtOH and hot water (1:10) for 24 hours for 3 times and solvent was removed under reduced pressure to obtain the extract as a brown gum. The ultrasonic assisted extraction condition was dried fruits *A. marmelos* mixed with EtOH and water at room temperature (1:10). The ultrasonic probe was pulsed on 10 seconds and stopped for 5 seconds and total extraction time 15 minutes for 5 times. The extract was filtrated and concentrated using rotary evaporator to receive brown viscous extract [8].

Determination of total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method [9]. All samples were prepared and

measured in triplicate. The standard curve of gallic acid was evaluated and used as standard compound. Test sample (100 µL) was mixed with EtOH for dilution. After that the reaction mixture was kept for 6 minutes after the addition of 20 µL of Folin-Ciocalteu phenol reagent. After incubation period, 100 µL of Na₂CO₃ was filled and mixed completely. The reaction mixture was incubated in dark at room temperature for 90 minutes. The mixture was measured absorbance at 765 nm. The TPC was reported in terms of mg GAE/g crude extract.

Antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging capacity of *A. marmelos* extract was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [10]. The DPPH stock solution was prepared from DPPH 6.3 mg in absolute EtOH 40 mL to obtain concentration 400 µM. The preparation of working DPPH was mixed stock solution 20 mL and absolute EtOH (1:1). The absorbance of the DPPH solution was measured at 517 nm. The DPPH solution was diluted by EtOH to obtain the absorbance between 0.9-1.1. Furthermore, the standard and extract of various concentrations (1,000, 800, 400, 200, 100 and 50 µg/mL) 20 µL were added in 96 well plates and then DPPH solution 180 µL was mixed. The mixture was shaken vigorously and allowed to stand for 30 minutes in the dark at room temperature. The absorbance of the solutions was determined by a spectrophotometer at 517 nm. L-ascorbic acid was used as a control and evaluated for triplicate. The percentage inhibition of free radical DPPH was calculated according to the equation:

$$\% \text{ Inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Ferric-reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay is based on the ability of antioxidants to reduce Fe³⁺ to Fe²⁺ in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe²⁺-TPTZ complex which exhibited the maximum absorption at 593 nm [10]. The mixture of sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM Ferric chloride solution (10:1:1) was FRAP reaction solution. The solution was prepared fresh and warmed at 37°C in a water bath before use. Samples were prepared in the concentration 50, 100, 200, 400, 800 and 1,000 µg/mL, respectively then added to 3 mL of FRAP reagent. The reaction mixture was incubated at 37°C for 30 minutes and determined absorbance at 593 nm. Fresh working solutions of FeSO₄ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample is calculated from the linear calibration curve and expressed as µM/g crude extract.

Results and discussions

Total phenolic content

The determination of total phenolic content using Folin-Ciocalteu method. The absorption of samples was compared with standard curve. The results showed the ultrasonic assisted extraction provide higher total phenolic content than extraction by maceration of both solvents. Water extraction found total phenolic content between 56.80±0.01 – 70.44±0.03 mg GAE/g crude extract. In addition, the ethanolic extract exhibited total phenolic content in the range of 56.14±0.02 – 79.75±0.05 mgGAE/g crude extract. A comparison of extraction time, UAE with water and EtOH showed 15 minutes provided higher phenolic content than 30 minutes according to the longer extraction time might affect to phenolic constituents in extract. Moreover, *A. marmelos* fruits extract by UAE contained TPC about 64.94-79.75 mg GAE/g extract while maceration showed TPC between 56.18-56.80 mg GAE/g extract

(figure 1). The ultrasonic wave can be absorbed by chemical bonds in sample and possible to break or generate new bonds.

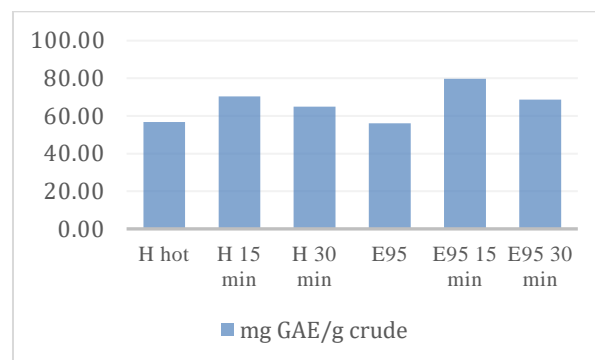


Figure 1 Total phenolic content of *A. marmelos* fruits extract

Table 1 The percent inhibition of water and ethanolic extract of *A. marmelos* fruits

Condition	% Inhibition	
	Water extract	EtOH extract
Hot water	28.09	-
95% EtOH	-	28.40
Ultrasonic 15 min	32.17	37.53
Ultrasonic 30 min	31.61	39.50

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity screening of *A. marmelos* fruits extract using DPPH radical scavenging method. The extract exhibited percent inhibition between 28.09-39.50%. The DPPH activity of water extract showed the highest inhibition value at 32.17% in ultrasonic condition 15 minutes whereas the highest inhibition of ethanolic extract was ultrasonic condition 30 minutes (table 1).

Ferric-reducing antioxidant power assay

The FRAP method has been selected to investigate antioxidant activity based on their reduce power of TPTZeFe (III) complex to the TPTZeFe (II) complex. The complex of Fe²⁺ exhibited maximum adsorption band at 593 nm. The results showed the highest antioxidant was the extraction with hot water at 355.74 µM/g crude extract. Crude water showed higher FRAP values than EtOH extract with FRAP values between 287.26-355.74 and 259.96-302.46 µM/g crude extract. The extraction time of ultrasonic condition showed the corresponded time at 15 minutes with TPC to obtain higher values of phenolic compounds (figure 2).

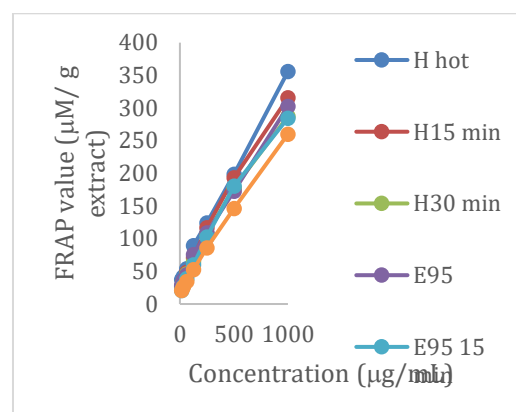


Figure 2 FRAP values of *A. marmelos* fruits extract

The maceration method spent longer extraction time (3 days) and obtained less total phenolic content due to the extraction mechanism was like dissolved like which depend on polarity of solvent. UAE provided high content of total phenolic compounds according to ultrasonic wave break cell wall in plant materials so the constituents were eluted. A suitable time for extract by

UAE around 15 minutes received more phenolic compounds whereas excess extraction time may affect to composition of constituents. The extraction time of UAE is less than maceration around 15-30 minutes for each time.

Conclusion

The investigation of *A. marmelos* fruits extract using maceration and ultrasonic assisted extraction. The ultrasonic assisted extraction at 15 minutes provided the highest total phenolic content. The antioxidant activity of extract, ethanolic extract using UAE 30 minutes exhibited the highest percent inhibition values at 39.50% in DPPH assay while hot water extract by maceration showed the highest antioxidant activity using FRAP assay at 355.74 µM/g crude extract. The finding of the present study suggested that UAE enhanced total phenolic content of crude extract and reduce extraction time than maceration. This method can be applied for the extraction of other kind of plants.

Competing Interests

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Funding

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Author contributions

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A Cross-sectional Survey of Traditional Chinese Medicine Constitution in Thailand's Type 2 Diabetic Population

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ABSTRACT

Introduction: This paper uses "Traditional Chinese Medicine Constitution Classification and Judgment Table" to conduct a cross-sectional survey and research the traditional Chinese Medicine constitution in Thailand's type 2 diabetic population.

Objective: To examine the TCM constitution of patients with type 2 diabetes in Thailand.

Methods: This study selected 424 Type 2 diabetes patients from January 2021 to July 2021 in Roi Et City, Thailand, Public Health Service Center, Traditional Chinese Medicine Outpatient Department, and Inpatient Department, Nakhon Ratchasima City, Thailand, Hua Laem Community, Don Thabuang Community, using "Traditional Chinese Medicine Constitution Classification and Judgment Table" for investigation, using Microsoft Excel to collect the general data and the distribution characteristics of TCM constitution, and SPSS 23 software for analysis.

Results: Among 424 people with type 2 diabetes in Thailand, Female patients were 58.96%, and Male patients were 41.04%. The age distribution is at most between 46- and 55 years old. For general data, Patients with a high BMI were 55.42%. The most significant number of cases in the distribution of TCM constitution is "Yin deficiency constitution," 30.2%. The highest distribution of syndrome types was "Qi and Yin deficiency syndrome," 39.7%. The difference was statistically significant ($P < 0.05$).

Conclusion: "Yin deficiency constitution" and "Qi and Yin deficiency syndrome" is found the most in Thai type 2 diabetes patients. Gender, age, education level, diet, and BMI are the factors that affect the formation of the biased constitution in patients with type 2 diabetes.

Keywords: *type 2 diabetes; TCM constitution; cross-sectional survey; Thailand*

Introduction

糖尿病是以慢性血糖水平升高为主要特征的代谢性疾病[1],全球糖尿病的整体发病率正在迅速增长。The Diabetes Federation (IDF)[2]发布数据表明,2015年全球糖尿病的患病人数高达4.15亿,且将在2040年达到6.42亿,这一数据让人触目惊心。而有相当大部分的糖尿病患者生活在低收入国家,目前在中国国家的糖尿病患者人数已然居于世界首位。

就目前中国糖尿病的流行趋势来说,相比于西方国家要更为严峻。1980年中国糖尿病的患病率仅为0.67%[3],到2010年,在中国全国范围内开展的采取ADA诊断标准的流行病学调查中显示,中国成年人中糖尿病患病率已高达11.6%[4]。

据泰国《世界日报》[5]2013年6月26日消息,泰国卫生部长巴迪医师表示,泰国糖尿病的患病率仅为0.61%,进10年来泰国因患糖尿病入院治疗的人数增加了4倍,目前泰国患糖尿病人数高达480万人,平均每天死亡200人。对比与西方人群,中国

和泰国糖尿病患病率急剧上升的可能原因大致包括人口老龄化, 生活方式的改变, 糖类摄入增加而蔬果类摄入减少, 超重和肥胖比例增加等[6]。

中医学对糖尿病的认识已有三千多年的历史。将糖尿病归属于中医“消渴”、“消瘴”、“肺消”、“膈消”、“消中”等范畴。消渴病是以多饮、多尿、多食、形体消或尿有甜味为特征的疾病“三多一少”, 证候特征不同而分为上消、中消、下消以口渴多饮为主者为上消, 消谷善饥为主者为中消, 小便多而频或浑浊为特点者为下消。但是临床上有一部分患者并无明显“三多一少”症状, 需要深度的研究, 为了更多了解糖尿病的症状, 体质, 证型等方面, 并且为临床提供更为有效的预防疾病和为今后中国和泰国健康管理工作及中国和泰国治未病服务提供坚实的基础。

Methodology

1. 临床资料

1.1 研究对象

病例均来源于 2021 年 01 月到 2021 年 07 月间, 以居住于泰国, Roi Et 市, Public Health Service Center, 中医科门诊及住院部地区; 泰国, Nakhon Ratchasima 市, Hua Laem 社区; Don Thabuang 社区, 18-65 周岁人群, 西医和中医科门诊及住院部诊治, 明确诊断为 2 型糖尿病人群及临床资料完整的患者为研究对象, 共 424 例作为调查样本。

1.2 诊断标准

1.2.1 中医诊断标准: 参考 2011 年《糖尿病中医防治指南》。

1.2.2 西医诊断标准: 参考 2020 年《中国 2 型糖尿病防治指南》。

1.2.3 中医体质分类与判定标准: 参考 2009 年《王琦教授九种中医体质分类判定标准》。量表由平和质、气虚质、阳虚质、阴虚质、痰湿质、湿热质、瘀血质、气郁质、特禀质量表构成。

1.3 纳入标准

(1) 泰国国籍者; (2) 无限性别; (3) 年龄为 18-65 周岁; (4) 明确诊断为 2 型糖尿病的患者; (5) 患者能配合研究者完成中医体质及有关病史资料的完整采集; (6) 临床检查资料完整。

1.4 排除标准

(1) 不符合诊断标准和纳入标准者; (2) 病情危重的病例: 如肿瘤与白血病围化疗期, 肾功能不全, 肝硬化以及药物影响 (如长期服用利尿剂, 硝苯地平, 复方降压片, 比嗪酰胺, 普洛萘尔, 小剂量阿司匹林, 抗结核药) 等; (3) 患者不配合或不能配合研究者完成中医体质及有关病史资料的完整采集; (4) 临床检查资料不完整。符合上述项目中任意一项者, 即予排除。

1.5 易除病例标准

不完整调查问卷, 且不能判断中医体质类型。

2. 研究方法

严格按照纳入病例标准, 排除病例标准及易除病例标准选择 2021 年 01 月到 2021 年 07 月间, 以居住于泰国, Roi Et 市, Public Health Service Center, 中医科门诊及住院部地区; 泰国, Nakhon Ratchasima 市, Hua Laem 社区; Don Thabuang 社区, 18-65 周岁人群, 西医和中医科门诊及住院部诊治, 明确诊断为 2 型糖尿病人群及临床资料完整的患者为研究对象, 共 424 例作为调查样本, 进行调查, 调查表来源 2009 年《王琦教授九种中医体质分类判定标准》, 包括一般情况、危险因素、中医体质、中医证型及调查员填写部分等, 最后采用 Microsoft Excel 软件进行数据管理, 建立本课题相关数据库, 在全部记录输入完成后, 通过调入用 IBM SPSS Statistics 23 软件进行分析。

2.1 样本量的确定

依照多因素分析的相关理论, 样本数量是变量的 5 至 10 倍[15]。《中医体质分类与判定表》纳入变量为 67 个, 而样本量大于 335 可判定其符合标准, 本文选取样本量为 424 例。

2.2 调查质量控制及再调查的实施

调查采用横断面调查方法, 调查员母语为泰语, 具有本科及以上学历中医学相关教育背景者。为保证研究资料的准确性, 完整性以及可靠性和真实性, 制订了问卷中每环节的操作规则。问卷发放前对研究人员进行调查方法以及相关知识点的培训, 进一步规范了调查流程。在整个研究过程里, 问卷的填写内容需根据患者自身以及研究人员辅助填入, 禁止擅自更改患者填写内容。调查问卷中的患者一般资料由患者本人写入, 而脉象以及体征等部分由研究人员现场检查后进行写入。问卷内容的写入需保持完整认真的态度, 写入完毕后由专门的研究人员针对问卷填写情况进行数据录入、分析, 确保问卷内容的可靠以及完整性, 如果在填写过程中确实需要更改需详细写入更改原因并标明日期。研究人员需要对纳入对象的基本资料进行记录以此确保回访等后续过程的实施。

2.3 研究工具

调查问卷包括一般人口学资料, 体质量表和医生判断填写三个部分。人口学资料涵盖性别, 职业, 年龄, 身高, 吸烟饮酒情况, 婚姻以及体重和文化等。

中医体质分类与判定标准: 泰文版中医体质量表是按照中文版中医体质量表条目形成。参考 2009 年《王琦教授九种中医体质分类判定标准》。量表由平和质、气虚质、阳虚质、阴虚质、湿热质, 气郁质, 特禀质以及瘀血质和痰湿质二级量表组成。

2.4 判定方法

在被调查者已完成填写《中医体质分类与判定表》的全部问题后, 调查者收集后进行计算, 计算方法是每一个问题按 5 级评分, 计算原始分及转化分, 依标准判定体质类型。

原始分 = 各个条目的分会相加。

转化分数 = [(原始分-条目数)/(条目数×4)]×100

平和质为正常体质，其他 8 种体质为偏颇体质。判定标准如下。

- 平和质：转化分 ≥ 60 分 → 是；
- 其他 8 种体质转化分均 < 30 分 → 是；
- 转化分 ≥ 60 分 → 基本是；
- 其他 8 种体质转化分均 < 40 分 → 基本是；
- 不满足上述条件者 → 否。
- 偏颇质：转化分 ≥ 40 分 → 是；
- 转化分 30~39 分 → 倾向是；
- 转化分 < 30 分 → 否。

2.5 数据处理与统计学分析

通过对收回的调查资料进行人工筛选并检查，对受试者的一般情况答空一项或者问卷条目中空出一项，共空出两项及以上的问卷均进行排除。将有效问卷录入 Microsoft Excel 表格，建立数据库，以保证数据的准确性。统计分析采用 IBM SPSS Statistics 23 软件，采用 χ^2 (卡方)、Kruskal-Wallis、Pearson χ^2 检验比较各体质组成比的差异及中医体质的影响因素进行分析。

Results

1. 泰国 2 型糖尿病总人群的中医体质分布情况

本研究在泰国对 2 型糖尿病人群共纳入 424 名为研究对象，将 424 例调查问卷根据回答《中医体质分类与判定表》中的全部问题，每一问题按 5 级评分，计算原始分及转化分，依标准判定体质类型。

(为了便于偏颇体质按其相应体质计算)，其体质分布情况如图 1 所示。

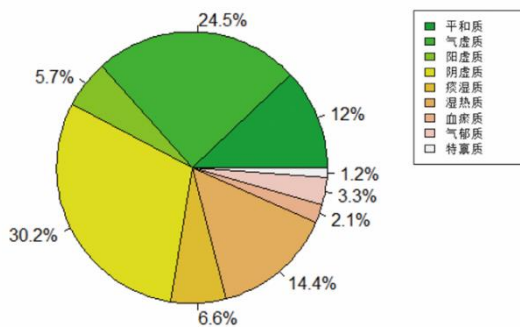


图 1：研究对象的体质分布情况饼图

从图 1 在泰国对 2 型糖尿病人群，424 例接受调查者中体质分布情况可知，体质分布情况的例数最多为“阴虚质”共 128 例 (占 30.2%)；其次为“气虚质”共 104 例 (占 24.5%)；“湿热质”共 61 例 (占 14.4%)；“平和质”共 51 例 (占 12%)；“痰湿质”共 28 例 (占 6.6%)；“阳虚质”共 24 例 (占 5.7%)；“气郁质”共 14 例 (占 3.3%)；“血瘀质”共 9 例 (占 2.1%)；体质分布情况的例数最少为“特禀质”共 5 例 (占 1.2%)。

本次研究体质频率依次为 阴虚质 > 气虚质 > 湿热质 > 平和质 > 痰湿质 > 阳虚质 > 气郁质 > 血瘀质 > 特禀质。

2. 泰国 2 型糖尿病人群的性别分布情况及不同体质研究对象的性别分布情况

2.1 性别分布情况

在泰国对 2 型糖尿病人群，424 名研究对象中，有 174 名 (占 41.04%) 为“男性”；有 250 名 (占 58.96%) 为“女性”，具体情况如图 2 所示。

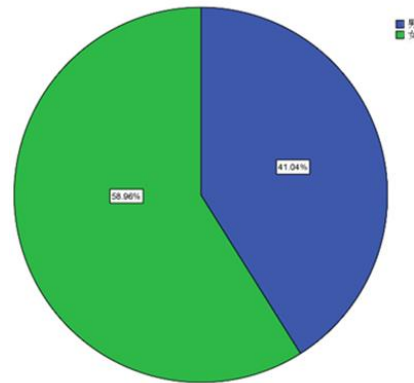


图 2 全体研究对象的性别分布情况

2.2 不同体质研究对象的性别分布情况

不同体质研究对象的性别分析结果显示，不同体质的研究对象性别构成存在差异 (P=0.002)。见表 1。

表 1 不同体质研究对象的性别分布情况(n (%))

性别	平和质	气虚质	阳虚质	阴虚质	痰湿质	湿热质	血瘀质	气郁质	特禀质	P*
男	21(41.18)	32(30.77)	4(16.67)	60(46.88)	8(28.57)	34(55.74)	7(77.78)	6(42.86)	2(40.00)	0.002
女	30(58.82)	72(69.23)	20(83.33)	68(53.12)	20(71.43)	27(44.26)	2(22.22)	8(57.14)	3(60.00)	

*注：经 Fisher 确切概率法进行统计学分析。

3. 泰国 2 型糖尿病总人群的一般资料及中医证型分布情况

(1) 年龄分布情况：研究对象中年龄最大为 65 岁，年龄最少为 20 岁，年龄分布情况的例数最多在“46-55 岁”占 48.58%。(2) 血型分布情况：血型分布情况的例数最多为“O 型”占 44.81%。(3) BMI 分布情况：BMI 分布情况的例数最多为“BMI 偏高”占 55.42%。(4) 居住地类型分布情况：居住地类型分布情况的例数最多为“农村”占 79.25%。(5) 受教育程度分布情况：受教育程度分布情况的例数最多为“本科及以上”占 39.86%。(6) 职业分布情况：职业分布情况的例数最多为“农民”占 26.93%。(7) 职业类型分布情况：职业类型分布情况的例数最多为“都是体力劳动者和脑力劳动者”占 56.37%。(8) 轮班分布情况：轮班分布情况的例数最多为“不轮班”占 82.08%。(9) 夜班分布情况：夜班分布情况的例数最多为“不是夜班”占 94.34%。(10) 糖尿病家族史情况：糖尿病家族史情况的例数最多为“有糖尿病家族史”占 61.56%。

(11) 病程时间分布情况：病程时间分布情况的例数最多为“3-4 年”占 42.92%。(12) 诊治分布情况：患者有 100% 进行医疗诊治以及有定期就诊。(13) 患者对血糖控制分布情况：本次研究结果显示患者对血糖控制情况不好（未达到标准）占 53.54%。(14) 患者对 2 型糖尿病及糖尿病并发症的了解分布情况：本次研究结果显示患者对 2 型糖尿病及糖尿病并发症的了解分布情况为“不太了解”占 63%。(15) 合并疾病分布情况：研究结果显示合并高血压、高脂血症和周围神经病变比较多。(16) 口味分布情况：口味分布情况的例数最多为“喜甜味（甜食）”占 41.9%。(17) 饮食类型分布情况：饮食类型分布情况的例数最多为“喜油炸食品”占 32.5%。(18) 喝酒分布情况：喝酒分布情况的例

数最多为“不喝酒”占 60.61%。(19) 吸烟分布情况：吸烟分布情况的例数最多为“不吸烟”占 75.71%。(20) 体育锻炼情况：体育锻炼情况的例数最多为“不锻炼”占 71.93%。(21) 睡眠分布情况：睡眠分布情况的例数最多为“不熬夜”占 78.30%。(22) 入睡时间分布情况：入睡时间分布情况的例数最多在“9 点~10 点”占 45.52%。(23) 睡眠持续时间分布情况：睡眠持续时间分布情况的例数最多在“5~6 小时”占 54.72%。(24) 性格及精神状态分布情况：性格及精神状态分布情况的例数最多为“平和开朗”占 54.7%。(25) 证型分布情况：证型分布情况的例数最多为“气阴两虚证”占 39.7%；其次为“痰热互结证”占 19.10%；证型分布情况的例数最少为“阴阳两虚夹痰浊证”占 2.36%^[7-14]。其全体研究对象的证型分布情况及不同体质研究对象的证型分布情况如图 3 和表 2 所示。

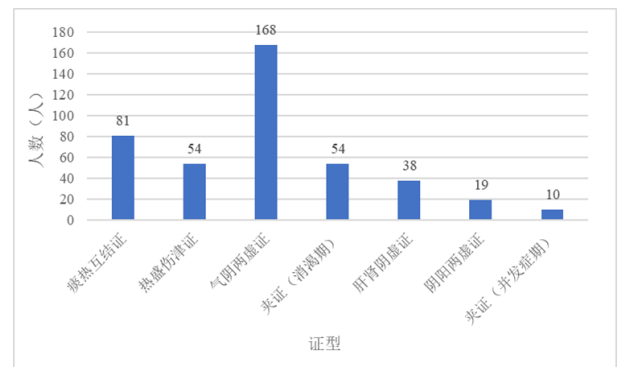


图 3 全体研究对象的证型分布情况

表 2 不同体质研究对象的证型分布情况(n (%))

证型	平和质	气虚质	阳虚质	阴虚质	痰湿质	湿热质	血瘀质	气郁质	特禀质	P*	
消渴期	痰热互结证	12 -23.53	6 -5.77	0 0	3 -2.34	11 -39.29	43 -70.49	5 -35.71	1 -7.14	0 0	<0.001
	热盛伤津证	17 -33.33	7 -6.73	0 0	15 -11.72	10 -35.71	3 -4.92	2 -14.29	0 0	0 0	
	气阴两虚证	9 -17.65	71 -68.27	0 0	83 -64.84	1 -3.57	2 -3.28	0 0	2 -14.29	0 0	
	夹证	6 -11.76	9 -8.65	0 0	23 -17.97	2 -7.14	13 -21.31	0 0	1 -7.14	0 0	
并发症期	肝肾阴虚证	4 -7.84	6 -5.77	0 0	10 -71.43	7 -29.17	2 -7.14	1 -7.14	4 -3.13	4 -80	
	阴阳两虚证	0 0	3 -2.88	11 -45.83	2 -3.92	1 -3.57	0 0	1 -7.14	0 0	1 -20	
	夹证	0 0	1 -3.57	6 -25	2 -1.92	1 -1.96	0 0	0 0	0 0	0 0	
	夹证	0 0	1 -3.57	6 -25	2 -1.92	1 -1.96	0 0	0 0	0 0	0 0	

注：经 Fisher 确切概率法进行统计学分析

Conclusion

4.1 泰国 Nakhon Ratchasima 市和 Roi Et 市两个市的 2 型糖尿病患者，中医体质分布情况的例数最多

为“阴虚质”，归纳总结高发易感体质为：湿热体质和阴虚质以及气虚质，平和质比较多。

4.2 泰国 Nakhon Ratchasima 市和 Roi Et 市两个市的 2 型糖尿病患者，中医证型分布情况的例数最多为“气阴两虚证”，归纳总结高发易感证型为：气阴两虚证、痰热互结证、热盛伤津证、气阴两虚夹痰浊证和肝肾阴虚证比较多。

4.3 性别、年龄、文化程度、饮食、BMI 是影响 2 型糖尿病患者偏颇体质形成的因素。其中，本科及以上、初中及以下、高中文化程度、为气虚体质危险因素，性别（男性）为气虚体质保护因素；嗜甜、嗜辣的饮食习惯是阴虚质的危险因素，BMI 是阴虚质的保护因素；BMI、偏甜的饮食习惯是痰湿质的危险因素；BMI 是阳虚质的保护因素；年龄是血瘀质的危险因素。

本研究应用现行的《中医体质分类与判定表》对泰国 2 型糖尿病人群横断面调查研究，而从样本分析中医体质对泰国 2 型糖尿病人群的影响，有助于推动中医体质学的国际化发展，初步探索泰国 2 型糖尿病人群的中医体质要素和特征，为中医干预提供依据，并且为临床提供更为有效的预防疾病和为今后中国和泰国健康管理工作及中国和泰国治未病服务提供坚实的基础。

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Antiglycation, α -glucosidase, and α -amylase Inhibitory Activity of Guava Leaf Hydrosol

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ABSTRACT

Introduction: Guava leaf oil (GLO) is one of the economic products obtained from the hydrodistillation process. It is widely used in the cosmetic, pharmaceutical, and nutraceutical industries. In this process, residual water (hydrosol) is generally drained out as effluent. There is no information available regarding this hydrosol's bioactivity for health.

Objective: This study aimed to evaluate the antiglycation and inhibitory activity of α -glucosidase and α -amylase enzymes which are related to diabetes mellitus.

Methods: Hydrosol used in this study was obtained from the essential oil production of guava leaves using the hydrodistillation process. This process was performed at guava leaves to water ratio of 1:10 (w/v) at 100 °C for 2 hours. The obtained hydrosol was then freeze-dried to obtain the hydrosol powder. Phenolic content (TPC) of hydrosol powder was determined using both Folin-Ciocalteu's reagent and HPLC methods. Inhibition of glycation, α -glucosidase, and α -amylase of hydrosol was also evaluated.

Results: The hydrosol yielded 5.4±0.8 % w/w of fresh leaves. TPC of the hydrosol was 134.3±3.6 mg of gallic acid/ g of hydrosol powder. The Antiglycation (IC₅₀) of the hydrosol was 9.6±1.3 µg/mL, which showed higher efficacy than aminoguanidine. Inhibition of the hydrosol (IC₅₀) against α -glucosidase, and α -amylase was 413.3±10.7 and 596.6 ±4.6 µg/mL, respectively.

Conclusion: Hydrosol byproduct obtained from essential oil distillation of guava leaves should be promising candidates for hypoglycemic functional product and antiaging ingredient.

Keywords: guava leaf; hydrosol; α -Glucosidase; phenolic compounds; glycation

Introduction

The nonenzymatic reaction between reducing sugar and the amino group of proteins spontaneously initiated the high reactive carbonyl radicals (the glycated products) in the body [1]. Accumulation of these glycated products induces irreversible skin collagen [2] and the pathologies such as Alzheimer's [3], chronic heart failure [4], and diabetes [5]. Carbohydrate hydrolytic enzyme such as α -glucosidase and α -amylase is one of the main causes to generate glycated products [6]. Several drugs have been widely used to inhibit two enzymes and glycated products. However, these drugs displayed side effects such as liver disorders [7,8].

Therefore, the natural product possesses both these enzymes' inhibitory and antioxidant activity and has been increasingly studied to use as an alternative source) [9]. Among medicinal plants, *Psidium guajava* is one of the interesting natural sources for solving these problems [10].

Psidium guajava L. (guava) is a plant of the Myrtaceae family. This is one of the most effective plants used in folk medicine for the treatment of various ailments such as gastroenteritis, and diarrhea rheumatism [11]. Guava leaf extract obtained from different solvent extraction such as chloroform, ethanol, and water also inhibited α -glucosidase and α -amylase

including displayed antioxidant activity [10]. Many phenolic compounds of this plant such as gallic acid, ellagic acid, and quercetin have been qualified and quantified [12,13]. These phenolics are related to the biological activities of guava leaves. In addition, several studies report on guava leaf oil used extensively, especially in the pharmaceutical, cosmetic, food, and perfume industries due to its chemical components attributed to a variety of effective biological activities, such as anticancer, antioxidant, anti-inflammatory, and antimicrobial [14,15]. However, residual water (hydrosol) is generally drained out as effluent in the hydro distillation of essential oil production. In recent years, many studies reported that polar volatile compounds and other secondary metabolites of the plant could interact with water via hydrogen bonds during the process of essential oils extracted from aromatic plants [16,17]. To water extract of guava leaves gave inhibitory activity against α -glucosidase and α -amylase [10] and information on the guava leaf hydrosol is no information. Therefore, the determination of the hydrosol activity against these enzymes is interesting. This study aimed to evaluate phenolic content and determine some phenolic compounds such as gallic acid, ellagic acid, and quercetin of this hydrosol. In addition, antiglycation and enzyme inhibition of α -glucosidase and α -amylase were also evaluated.

Methodology

Chemical reagents

The chemical reagents included p-nitrophenyl- α -glucopyranoside (4-pNPG), soluble starch from potatoes, α -glucosidase from *Saccharomyces cerevisiae*, α -amylase from *Aspergillus oryzae* were purchased from Sigma–Aldrich (St. Louis, MO, USA). Hydrosol is obtained from essential oil distillation of guava leaves at a weight ratio of guava leaves to water equal to 1:10 at 100 °C for 2 h. The hydrosol was further freeze-dried to obtain the powder and kept at 4 °C for further use.

Phenolic compound analysis

Phenolic compounds existing in the hydrosol that is obtained from hydro-distillation of guava leaves were identified using a reported method [12,13] with some modification. Briefly, HPLC analyses were performed using a UV detector set at 280 nm. Acetic acid (2% v/v) and acetonitrile (50%, v/v) were used as gradient solvents at a flow rate of 1 ml/min. Hydrosol was dissolved in absolute ethanol and filtrated through a 0.45 μ m Millipore filter prior to injecting into the HPLC system. The sample was analyzed in triplicate. Phenolic standards of interest such as gallic acid, ellagic acid, and quercetin were used for the identification of phenolic compounds that existed in the hydrosol. The identified phenolic compounds were quantified based on their peak area and compared with calibration curves obtained with the corresponding standards and then expressed as mg/g of extract.

Determination of total phenolic content

The total phenolic content of the hydrosol was determined by the Folin–Ciocalteu method according to described by the reported method [13] with some modifications. Briefly, the hydrosol was dissolved in absolute ethanol to a final concentration of 1 mg/mL prior to the test. An aliquot of 50 μ L of the hydrosol solution was mixed with 50 μ L of Folin–Ciocalteu reagent. After that, 100 μ L sodium carbonate (2% w/w) was added. The mixture was allowed to stand for 1 h at room temperature. The absorbance was measured spectrophotometrically at 750 nm. The experiment was done in triplicate. Gallic acid was used as the standard for the calibration curve. The total phenolic content of the sample was expressed as gallic acid equivalent (GAE) to 1.0 g of extract.

In vitro α -glucosidase inhibitory activity

Inhibition of α -glucosidase was determined as the reported method [9] with some modifications. Briefly, the hydrosol solution was mixed with 4-pNPG (0.25 mM) and α -glucosidase solution from *S. cerevisiae* (0.1 U/mL). Then, the reaction mixture was incubated at 37 °C for 20 min. After incubation, the enzyme activity in the reaction was stopped by adding 0.50 M Na₂CO₃. The release of the p-nitrophenolate group was measured at 405 nm using a UV/Vis spectrophotometer.

In vitro α -amylase inhibitory activity

Inhibitory activity of the guava hydrosol against α -amylase was determined according to the reported method [18] with some modifications. Aliquots of the hydrosol were mixed with soluble starch (1%, w/v) in 20 mM PBS (phosphate buffer saline, pH = 7.4). The mixture was incubated at 37 °C. After 10 min, α -amylase (1.5 U/mL) was added, and the mixture was incubated at 37 °C for a further 10 min. Then, 3, 5-dinitrosalicylic acid (DNS) solution was added and incubated at 100 °C for 5 min to completely inactivate the enzyme. The absorbance was measured at 540 nm using a spectrophotometer. Acarbose was used as a positive control.

In vitro antiglycation activity

The antiglycation activity of the hydrosol was determined according to the described assay [19] with some modifications. Briefly, Initially, the guava hydrosol, collagen (45 μ M), glucose (200 mM), and fructose (200 mM) were added in phosphate buffer (20 mM, pH 7.4) containing 100 mM sodium chloride. 0.31 mM sodium azide was added to achieve an aseptic condition. Aminoguanidine was used as a positive control. Reaction systems were incubated in the dark for 7 days. Antiglycation activity was quantified using Spectro fluorophotometer (λ_{ex} = 370 and λ_{em} = 440 nm). The experiment was conducted with three independent replicates. The IC₅₀ was calculated graphically, using a calibration curve, plotting the extract concentration versus the corresponding inhibition percentage.

Statistical analysis

To determine the statistical difference between means ($p < 0.05$), using SPSS statistical software package v. 22. Results were expressed as mean values \pm SD.

Results

Hydrosol obtained from hydrodistillation of a ratio of guava leaves: water at 1:10 (w/v) at 100 °C for 2 h yielded 5.4 \pm 0.8 %w/w of fresh leaves. Total phenolics of hydrosol powder was 134.3 134.3 \pm 3.6 mg gallic acid/g of hydrosol powder.

Phenolics of interest that existed in hydrosol powder were characteristic and its HPLC profile is shown in Figure 1. Peak no. 1, 2, and 3 represented gallic acid, ellagic acid, and quercetin at the retention time of 6.6, 40.2, and 60.3 min, respectively. The identified phenolic compounds were quantified based on their peak area and compared with calibration curves of standard compounds. The calibration curves of the standards were linear with R² values of 0.99 for gallic acid, ellagic acid, and quercetin. The amount of gallic acid, ellagic acid, and quercetin that exist in the guava hydrosol is shown in Table 1.

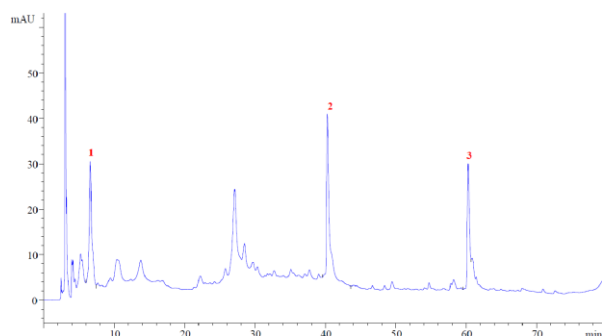


Figure 1 HPLC chromatogram of phenolic compounds existing in the guava hydrosol; (1) gallic acid; (2) ellagic acid; (3) quercetin.

Table 1 Phenolic content and phenolic compound existing in the guava hydrosol (GH).

Compound	Amount (mg/ g hydrosol)
Total phenolic content	134.3 \pm 3.6
Gallic acid	6.9 \pm 0.3
Ellagic acid	33.9 \pm 1.7
Quercetin	24.7 \pm 0.9

The guava hydrosol inhibited the mixture glycation of collagen, glucose, and fructose with an IC₅₀ value of 9.6 \pm 1.3 μ g/mL which was significantly higher efficacy than aminoguanidine. In addition, the guava hydrosol inhibited inhibit α -glucosidase and α -amylase at IC₅₀ values of 413.3 \pm 10.7 and 596.6 \pm 4.1, respectively.

Table 2 displays that ellagic acid inhibited both α -glucosidase and α -amylase with IC₅₀ of 37.4 \pm 1.4 and 194.4 \pm 2.8 μ g/ml which high efficacy than acarbose. Whereas gallic acid and quercetin inhibited only α -glucosidase with IC₅₀ of 848.3 \pm 4.3 and 82.0 \pm 1.8 μ g/mL, respectively.

Table 2 IC₅₀ values of guava hydrosol and the polyphenols exist in the guava hydrosol against α -amylase, α -glucosidase, and glycation of collagen-glucose-fructose.

Sample	Inhibitory activity (IC ₅₀ ; μ g/mL)		
	α -glucosidase	α -amylase	Glycation
GH	413.3 \pm 10.7 ^d	596.6 \pm 4.1 ^c	9.6 \pm 1.3 ^a
Acarbos	361.4 \pm 10.2 ^c	537.3 \pm 4.4 ^b	-
e			
AM	-	-	76.19 \pm 2.5 ^b
GA	848.3 \pm 4.3 ^e	-	-
EA	37.4 \pm 1.4 ^a	194.4 \pm 2.8 ^a	-
QCT	82.0 \pm 1.8 ^b	-	-

Note: GH= guava hydrosol; AM = aminoguanidine; GA= gallic acid; EA = ellagic acid; IC₅₀=concentration that inhibits 50% of the enzyme activity/glycation fluorescence. The different letters in the same column indicate a statistically significant difference ($P < 0.05$).

Discussion

The goal of this study was first to determine the phenolic content of the guava hydrosol due to previous studies reported that the amount of phenolic correlated with antiglycation activity and both enzyme inhibition [6,9,20]. Gallic acid, ellagic acid, and quercetin were chosen to examine their inhibitory effects on α -amylase and α -glucosidase since they are the most abundant polyphenol components in guava hydrosol. The pure polyphenol compound inhibited α -glucosidase more effectively than the hydrosol powder. This may be because the guava hydrosol had complicated phenolic compounds that prevented the action of individual polyphenol compounds attaching to the active sites of the enzymes or the enzyme-substrate complexes [10,21], so lowering their inhibitory impact. The inhibitory effect of polyphenol compounds on α -amylase may have been influenced not only by the overall polyphenols content but also by the identities and amounts of polyphenol compounds. Notably, unlike pure polyphenol compounds, quercetin and gallic acid exhibited no inhibitory impact on α -amylase. This may be owing to the different ring structures of polyphenol compounds. It has been shown that hydroxylation on the different positions of polyphenol rings can considerably alter their affinity for α -amylase, hence altering their inhibitory impact [22]. Regarding α -amylase inhibiting properties, the increased inhibitory impact of the guava hydrosol compared to the studied pure chemical components (gallic acid and quercetin) may be attributable to the effect of other unidentified components or the synergistic effect of some components that play a significant role in the enzyme's inhibition [23].

There is substantial evidence that the protein glycation process is one of the main factors contributing to the aging of the skin. AGEs build throughout the process of protein glycation and cause structural changes in proteins such as collagen. Natural and

manmade substances have been explored as AGE formation inhibitors. Compounds with antioxidant and photoprotective properties, such as gallic acid, ellagic acid, and quercetin, also prevent advanced glycation effectively [24]. In this work, the guava hydrosol inhibited the production of a glycation system made of collagen, glucose, and fructose very effectively, making it a great candidate for application as a natural treatment for degenerative disorders and aging. Prior to a prospective pharmaceutical application, however, additional research is required to determine the mechanism of action and the in vivo response in appropriate animals.

Conclusion

This work was first reported to evaluate the biological activity of hydrosol powder derived from essential oil distillation of guava leaves. The hydrosol powder expressed high activity against glycation, α -amylase, and α -glucosidase. The present activities can be attributed to bioactive phenols that exist in the hydrosol. The results suggest that guava hydrosol could be considered an alternative source of antiaging and antidiabetic ingredients.

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Research progress in the prevention and treatment of osteoporosis with exercise therapy

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ABSTRACT

Osteoporosis is a systemic bone disease. Osteoporosis is a systemic bone disease characterized by reduced bone mass per unit volume, degeneration of bone microstructure, thinning of bone density, reduction of bone trabecular number, widening of bone marrow cavity, and reduced bone strength, which are easy to fracture. Exercise therapy can effectively improve the bone mineral density of patients with osteoporosis and significantly intervene in the risk of osteoporosis. In addition, compared with drug therapy, exercise therapy has the advantages of economy and no adverse reactions, which attracts more and more people's attention. However, at present, patients and the public generally lack the correct understanding of exercise therapy, coupled with the characteristics of exercise itself (exercise mode, exercise intensity) and the influence of the patient's own conditions, the efficacy of exercise therapy in the practical application process has not been fully highlighted. This paper summarizes the effect of exercise therapy on osteoporosis, hoping to provide help for the clinical application of exercise therapy in the prevention and treatment of osteoporosis.

Keywords: *osteoporosis; exercise therapy; bone density*

Introduction

随着社会的发展,人口的增多,中国进入高速老龄化的阶段,加上其他药物的普遍使用,骨质疏松症(osteoporosis, OP)发病率的年平均增长率呈快速增长的势头,再加上骨质疏松性骨折的发病率和死亡率高,骨质疏松症已成为一个重要的健康问题。骨质疏松症是一种全身性骨骼疾病,病理特点为骨量低,骨组织微结构损坏。在正常的生理条件下,成骨细胞和破骨细胞、骨形成和骨吸收这两个过程精确地平衡,以保持骨骼健康。一旦这种平衡被破坏,最终就会发生骨丢失或骨形成过度,而如果骨丢失或骨吸收过多,就很容易导致骨质疏松症的出现。OP按病因可分为原发性和继发性,其中原发性OP最多常见,多发生于绝经后的女性以及老年人。最近研究发现 [1], OP 对人体的影响逐渐增多,患者出现了之前并没有被描述过的骨坏死和多发性骨质疏松性骨折,随着 OP 的不断发展,对人类的危害也在加大。OP 的危险因素包括种族、

体质问题、峰值骨量低、激素因素、某些药物(例糖皮质激素)的使用、吸烟、活动量减少、钙和维生素 D 摄入量低、缺乏日晒等 [2]。就性别而言,一项最近的研究发现,在 50 岁以上的中国人群中,女性 OP 的患病率为 29.0%,男性为 13.5% [3]。OP 患者最严重的并发症就是因低暴力导致的骨折,髌部骨折是 OP 最严重的并发症。当 OP 患者发生髌部骨折时,若治疗不当,死亡率将近 30%,是老年患者高致残致死率的主要原因之一 [4]。而且骨质疏松性骨折后治疗恢复需要投入大量的财力、物力,给患者家庭及整个社会带来沉重负担。

目前临床康复治疗 OP 主要包括宣传教育、药物治疗、运动疗法、作业疗法及康复工程等。目前,针对骨质疏松的治疗,药物疗法有其优势,但又有不可忽视的副作用,且费用高、时间长。因此,探索安全有效的治疗方法尤为重要。近年来有越来越多的学者认为缺乏运动是导致 OP 的主要原因之一 [5], 通过运动疗法能有效减缓骨质流失、

增加骨密度、降低跌倒率及骨折风险，而且运动疗法简单实用、副作用低、价格低廉。本文在前人基础上，对运动疗法的治疗效果进行系统的分析，为后续进一步基础研究及临床治疗提出理论依据和建设性建议。

1. 运动疗法

1.1 运动疗法的发展

运动疗法是指根据患者躯体功能障碍的情况，利用器械、徒手或患者自身力量，通过某些运动方式（主动或被动运动等），使患者获得全身或局部运动、感觉功能恢复的训练方法。目的是通过改善患者全身血液循环，使患者最大限度地恢复生活自理能力和劳动能力。我国最早有关于运动疗法的记载始于《黄帝内经》，之后不断发展完善，如果说《黄帝内经》是传统运动疗法的雏形阶段，那么《五禽戏》就是系统化的传统运动疗法 [6]。中国被公认为是世界上最早应用运动疗法的国家之一，早在公元前和公元初开始的“导引术”、“推拿术”、“气功”、“五禽戏”等已流传千年，并一直沿袭至今。只是，由于我国在运动疗法的发展过程中缺乏系统的归纳总结，我国许多传统运动疗法最终并没有形成完整的运动疗法体系，也没有成为国际主流的运动项目。国内外研究显示，接受本体感觉训练、平衡功能训练、水中运动治疗、行走与步态训练等运动疗法，不仅能够改善运动器官和心肺功能，更能提高神经系统调节能力，可有效预防或延缓认知功能的退化，改善患者预后[7]。近年来被广泛应用于认知功能障碍等疾病的临床干预治疗中。

1.2 运动疗法对骨质疏松症的影响

1.2.1 运动时应力刺激对骨骼的影响

机械应力的刺激可以维持骨骼系统的稳定，反之将引起骨代谢紊乱、骨量丢失及骨微结构退化，最终引起骨质疏松。长期卧床、失重等缺乏机械刺激的状态都将引起骨量显著丢失。人体不断进行着骨吸收和骨形成的骨重建过程，骨重建活动使骨组织不断更新，使微小的骨损伤得以修复，并向适应环境因素的方向变化。给机体施加应力负荷可以增加骨转换率，刺激成骨细胞生物活性，增加骨的重建和骨量的积累 [8]。目前，许多研究发现适宜的机械刺激有利于骨组织的形成和预防骨质疏松。有研究者 [9]研究股骨头内骨钙素、基因表达及蛋白质合成在机械应力刺激下不同时期动态变化，发现不适当的机械应力降低股骨头内骨钙素和蛋白质合成。曹盛楠等人[10]采用 Flexcell 体外细胞力学加载装置，施加 0.5Hz 频率、0.8% 应变率、每天 2 次、每次 30min 的间歇性牵张力，可促进小鼠成骨分化，表明了应力刺激对成骨细胞分化的促进作用。

1.2.2 运动调节机体内分泌而影响骨代谢

激素在保持骨结构和功能方面具有重要的意义，运动通过改变体内的激素分泌影响骨代谢。适

宜的运动可增加睾酮和雌激素的分泌而促进骨代谢，对女性而言，运动可以改善血清雌二醇，增加雌性激素的释放，从而起到预防作用，Pasqualini 等 [11]发现，长时间运动后，I型原胶原 N-端前肽和成骨细胞数量显著增加，而I型胶原 C-末端肽没有明显增加，运动抑制了骨吸收，刺激了骨形成，减缓了骨量流失。我们都知道，血清骨钙素 (BGP) 和碱性磷酸酶 (ALP) 作为检测骨成分的重要指标，其成分的变化能够准确的反映出骨在动态平衡中的状态，BGP 会在成骨细胞活跃时分泌量增加。有研究发现，运动干预后患者 BGP 和 ALP 水平显著上升，尿吡啶啉水平有所下降，腺苷三磷酸与腺苷一磷酸比例下降，激活蛋白激酶，即运动在促进成骨细胞活性的同时会抑制破骨细胞的分化 [12]，减少骨吸收。

在传导通路层面，运动可以影响微电位[13]，当运动增加时，产生的负性微电位与钙离子结合，从而促进骨的形成，对骨密度具有改善作用。在对 OPG/RANKL/RANK 轴与 OP 的关系研究中发现，其中骨保护素 (osteoprotegerin, OPG) 是由前成骨细胞/间质细胞分泌，核因子 κ B 受体活化因子 (receptor activator of nuclear factor- κ B, RANK) 是破骨细胞前体细胞膜受体，运动可以通过增加 OPG 和降低 RANKL [14]，从而抑制破骨细胞分化来促进骨骼健康。

1.2.3 运动可增强肌力

肌力对骨质疏松的影响不容忽视。研究显示随着年龄的增加，肌肉力量逐渐下降，骨质疏松症患者更是如此。肌力训练在 OP 患者中受到日益重视，肌力训练有助于稳定关节、增强平衡能力、减少损伤、提高生活质量。陈良慈 [15]研究发现骨质疏松症患者下肢肌力和平衡能力成正相关。此外，还有研究[16]表明运动训练可增强肌肉力量，提高平衡能力，减少跌倒的危险性，降低因骨质疏松引起的骨折发生率。

1.2.4 提高机体的平衡能力和灵活性

平衡是下肢和躯干通过协调运动来调整身体重心在支持面范围内的一种能力，即运动时中枢神经系统接受关节肌肉运动的本体感觉信号，从而对运动进行调节，以达到最平稳、最协调的运动模式，其中任何一个环节异常都可以导致平衡能力下降。运动训练可通过增加本体感觉输入、增强肌力、改善协调性和灵活性而加强平衡能力，有助于降低摔倒的风险和骨折的发生率。有研究发现运动具有能明显提高绝经后骨质疏松女性静态和动态平衡的作用 [17]。太极拳等运动方式也能提高中老年人的平衡能力，霍萧轻 [18]通过实验法，从单脚支撑能力、闭眼站立与睁眼站立保持稳定时人体中心点的左右及前后的位移和速度两个方面，去探讨太极拳对中老年人平衡能力的影响。该研究显示长期从事太极拳练习的中老年人，平衡能力明显较好。

1.2.5 运动促进骨骼关节的血循环和营养状态

运动可使机体血液重新分布, 肌肉、骨骼的血液供应量增加而改善骨骼的营养, 也可对关节软骨产生节律性挤压作用, 促进关节软骨的营养、延缓关节软骨的退变[19]。因此, 运动可改善骨骼和关节的营养代谢, 促进骨的重建和修复, 有利于 OP 患者的骨重塑及骨量恢复。

2. 不同运动方式对骨质疏松症的干预效果

OP 患者应加强体育锻炼, 能够增强患者骨强度, 改善肌腱和韧带的柔韧性和延伸性, 促进骨和肌肉的合成代谢, 增强患者的平衡能力。给予患者适当的运动锻炼, 可以有效改善患者的骨代谢, 实现骨生物学指标的优化, 延缓骨细胞衰老, 改善骨质疏松疾病的临床症状, 提升患者的生活质量。运动的种类繁多, 因为不同运动项目具有不同的特点, 根据训练方式的不同, 运动疗法又可分为有氧运动、抗阻训练、冲击性运动、负重训练等。不同的运动方式在临床实际应用中也表现出了不同的作用效果。

2.1 有氧运动

有氧运动是指在氧气供应充足的情况下进行的体育运动, 即在运动过程中, 人体吸入的氧气与消耗相等, 达到生理上的平衡状态, 并以大肌群节律性、重复性运动为特征。它主要分为快走、慢跑、游泳、爬山、跳舞等, 具备强度低、有节律、不间断、简单易行、容易坚持等特点。有氧训练可以促进骨形成和抑制骨吸收, 增加骨密度, 还可以改善心肺功能, 并对关节、肌肉、消化等多系统功能都有较好的促进作用。盖卓 [20]选取 40 只 6 月龄 SPF 级雌性 SD 大鼠, 随机分为假手术组 (Sham 组) 和实验组。实验组进行双侧卵巢切除手术, 达到雌激素缺乏导致骨合成代谢减弱的目的, 进而引发骨量减少导致骨质疏松, Sham 组切除卵巢旁等体积脂肪, 并予相应运动训练。研究表明有氧运动联合黄精多糖能显著改善骨小梁数量和疏松程度, 提高去卵巢大鼠骨密度和雌激素水平, 改善骨代谢指标, 提高 OPG/RANKL 比例, 并且联合干预的效果均优于单一干预。

2.2 抗阻训练

抗阻力运动又称力量运动或抗阻运动, 指的是肌肉在克服外来阻力时进行的主动运动, 可以有效延缓肌力下降、加速肌肉功能的恢复。主要包括克服弹性物体运动、负重抗阻运动和力量训练器械等, 目前被广泛应用于运动康复和肌肉萎缩训练 [21]。已经有大量的相关实验表明, 抗阻运动可以改善骨密度, 减少骨矿物质流失 [22], 增加如 1 型胶原蛋白氨基末端前肽等骨代谢产物含量。有研究表明, 抗阻训练联合其他运动方式可以显著提高骨量, 且研究表明抗阻训练可以提高绝经后女性的骨密度, 还能提高肌肉耐力和动态平衡能力 [23]。表

明了一定量的抗阻训练可以有效防治骨质疏松症。还有研究表明, 以上研究结果表明, 抗阻力训练有助于提高 OP 患者的肌力和精神状态, 对 OP 患者的肌肉功能水平的提高有促进作用。

2.3 冲击性运动

冲击性运动是指机体运动中受力点对机体的瞬间反作用力, 可根据是否需要站立分为高冲击力运动 (如跑步) 和低冲击力运动 (如游泳), 这种作用力刺激骨骼, 促使骨量增加。冲击性运动能提高骨密度和骨强度, 高冲击力运动比低冲击力运动更有利于达到理想的骨量峰值。有研究表明高强度家庭式冲击性运动对于低骨量的绝经后女性是安全可行的, 且能改善骨密度 [24]。因此应针对患者 OP 的严重程度, 选择不同强度的重击性运动项。

2.4 负重运动

负重运动是指通过器械或其他外物传达到骨骼的力对骨骼产生应力-应变刺激, 进而促进骨形成。张玮 [25]等通过总结分析不同运动治疗对围绝经期综合征的影响, 证实负重运动对骨密度的增长有效。不过由于负重运动强度过大, 对于大多数 OP 患者并不适宜, 因此运用负重运动是需考虑 OP 患者是否可以耐受。

2.5 振动训练

振动训练是一种新兴的非传统的训练方法, 让受试者借助于振动平台的振动刺激使人体产生适应性反应的外界干预方法。振动训练被认为是一种改善绝经后女性腰椎和股骨颈骨密度的有效方法, 且可以有效抵消老年人的肌肉力量损失, 降低跌倒率和骨折发生率。基础研究提示低频振动训增加股骨小梁密度能促进骨折愈合, 恢复骨骼力学性能 [26], 利于去卵巢大鼠骨质疏松改善和骨损伤愈合。

2.6 传统运动

我国的传统运动疗法注重维持身体健康的同时培育心神合一。该项运动注重顺应自然、阴阳平衡、身心合一, 从而达到修身养性的目的。太极拳、八段锦、气功便是几种常见的传统运动方式。已有研究表明, 传统运动兼具运动和心理训练的优点, 与单一的运动方式相比, 长期的传统运动对老年人身心健康的发展具有更佳的效益 [27]。

但需要注意的是, 不管是哪种运动方式, 对于患有严重 OP 的患者, 必须避免涉及躯干弯曲或旋转的动作以及超过患者自身能力范围外的大幅度、大强度的运动。

3. 不同运动强度对骨质疏松症的干预效果

运动强度是决定运动负荷的主要因素之一, 不同运动强度所表现出的效果是不同的。研究发现高强度下肢运动相较于中小强度运动, 一方面可以长时间 (一年左右) 维持骨矿物质和骨密度的稳定, 增加血清骨钙素的含量。另一方面也增加了机体的

灵敏度和力量,降低了跌倒骨折风险 [28],提高生活水平。

运动强度对不同人群影响是有区别的,同一运动项目,在接受范围内,强度越大其对人体骨质的影响越明显,有利于骨密度维持稳定。而人体作为一个复杂的个体,因为个体差异性,在制定运动处方的时候应该具有针对性,根据实际情况制定最恰当的运动强度。

4. 运动疗法结合其他方式对骨质疏松症的影响

4.1 运动疗法联合中药治疗干预骨质疏松症

银杏叶提取物作为中药治疗 OP 的新型药物,被越来越多专家所关注,仇业鹏等[29]将其与运动疗法联合干预 OP,发现血清雌二醇、丙二醛、血清骨钙、碱性磷酸酶和雌性激素等指标均得到提高,骨吸收和骨转换率降低,骨密度也得到维持。另外还有应用雷火灸[30]等中医手段与运动联合干预,在实际的应用中,运动疗法很好的融入到了中医的治疗方案中,两者结合表现出了明显的效果。

4.2 运动疗法联合针灸治疗干预骨质疏松症

针对脑卒中患者,应用温针灸联合运动疗法[31]来干预脑卒中后 OP,发现这一结合对骨代谢指标的提升具有明显效果,比单一手段更有作用,有效地减少了继发性骨折的发生。范萍[32]通过观察电针配合游泳运动对去卵巢骨质疏松症大鼠的骨组织形态学及 OPG 表达的影响,单用电针相比,电针配合游泳运动可有效改善去卵巢骨质疏松症大鼠的骨组织形态,并提高大鼠的骨密度、提升 OPG 的表达。

4.3 运动疗法联合西药治疗干预骨质疏松症

在骨质疏松症疾病的治疗中,治疗药物主要分为抗骨吸收药物、合成代谢药物及促骨矿化药物。药物疗法可以有效改善骨质疏松患者的临床症状,加快患者的康复,但是具有一定的副作用,而临床上采用运动疗法加上西药联合治疗逐渐发展为热门趋势。

在西医方面,有研究者将阿仑膦酸钠[33]与运动疗法结合,表现出了比单独干预更有益的效果,提高了患者的骨强度,这就警示要在正确的指引下进行西药药物加用治疗 OP,减少不必要的西药毒副作用的伤害。

4.4 运动疗法联合物理因子治疗干预骨质疏松症

物理因子治疗作为临床康复常用的治疗方法,其联合运动疗法对 OP 患者的功能恢复起到非常重要的作用,一方面相应的物理因子治疗能强化 OP 患者的神经功能,提高患者肢体的控制能力,增强力量,能是患者更有效的完成运动训练。另一方面,相应的物理因子治疗能缓解患者运动训练后的肌肉紧张,能使肌肉达到良好的恢复状态。有研究[34]探讨通过引导下脊神经后支阻滞联合运动疗法来治疗高龄骨质疏松性胸腰椎压缩性骨折

(OVCF) 患者,结果表明该联合疗法治疗高龄 OVCF 患者效果显著,可有效缓解疼痛,改善腰椎功能障碍,降低并发症发生率。因此,运动疗法联合物理因子治疗对于 OP 的功能恢复有良好的作用。

5. 运动疗法干预骨质疏松症可能存在的问题

虽然目前国内外已进行了大量的研究调查与实验,但对各种运动疗法的持续时间、强度、频率、适用对象、安全系数等方面尚未达成共识。此外,目前国内外在研究兼具科学性与实用性的个体化运动疗法领域仍处于薄弱阶段。运动疗法对 OP 的治疗起着积极有效的作用,但真正的实施过程中,又受到各种因素制约,难以达到预期效果。

5.1 运动处方的制定缺乏针对性

很多患者的 OP 往往是由于其他病症所引起的,因此,如何有针对性地制定干预措施也是另一大难题。研究表明[35],当 OP 患者患有癌症时,由于身体机能状态的原因,采用低强度运动就可以维持骨密度,起到较好的防治作用。人体的特异性及疾病不同,导致同一运动处方并不适应于所有患者,根据患者状态设计具有针对性的运动处方,可以保证有效治疗的同时,免受二次伤害。

5.2 运动疗法干预骨质疏松症评估方式及持久问题

运动疗法相较于药物治疗,短期干预难以产生显著变化,只有长期坚持才可以发挥最大的效果。很多老年人认为单一的运动处方限制了他们的运动类型,过于枯燥的项目和长期训练,会使他们感到乏味而不能坚持。目前 OP 最常用的检测方式是双能 X 线吸收法,但不足以解决日常人群的检测需求,并且难以检测骨骼内部结构的变化,一段时间的治疗结果难以直观地展现在患者眼前,患者难以在短时间内通过客观的数据去验证运动疗法对于自身的影响。总体来看,虽然很多运动实验干预 OP 患者取得了不错的效果,但由于个体差异,以及其他疾病影响,使得运动疗法的具体方案还需要大量临床试验验证以及针对性的运动处方,督促患者坚持完成,保证运动疗效。

Conclusion

综上所述,运动疗法是防治原发性骨质疏松症的一大手段,可以有效减缓骨质疏松的发展进程。从运动疗法的注意事项方面来说,OP 患者在选择运动项目前,需全面地了解自身病情;在运动量选择方面,要秉持着因人而异的原则,各个年龄阶段的患者,其运动量有着较大的差距;在运动强度方面,在不引发骨质疏松症患者疼痛或者疲劳的范围内,患者运动强度越大,越有利于骨密度的提升,改善患者骨质疏松症状;在频率方面,OP 患者的运动锻炼频率选择,主要以次日不感疲劳为主;在运动锻炼的阶段性问题,OP 患者需秉持

着“持之以恒”的原则，有规律、有计划地进行运动，建立良好的运动习惯，降低骨质的流失，从而改善患者的临床症状。

但作为一种疗法，运动疗法目前没有明确的适应症、禁忌症以及注意事项。同时，运动处方的制定既需要保证其安全性和有效性，也要兼顾个体的差异，故针对不同的年龄制定不同的运动处方，并需要遵循个体化原则，这些都还待进一步研究。另外，人们不仅仅是需要知道运动有益于人体健康，更加需要知道自己适合怎样的运动，每种运动的优劣，这就需要加大运动的教育力度和宣传，提高人们对运动的认知。最后，疾病的防治是一个长期坚持的过程，我们需要定期对疾病的进展进行了解，从而及时制定相应的运动方案，以达到最好的疗效。

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Discussion on the Application of Pharmacological Theory of Traditional Chinese Medicine in the Treatment of the Covid 19 Cases

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ABSTRACT

In clinical, there were limitations which the application of Traditional Chinese Medicine (TCM) using syndrome differentiation and treatment for novel epidemic disease. Meanwhile, modern scientific research gradually unravels the internal biochemical mechanism of the human body and analyzes the composition of Chinese herbs, and excavates and selects effective structures, and it has been confirmed that the combined application in treatment can expand the curative effect. Covid19 is a highly contagious modern epidemic disease, the only reliable way to diagnose patients is to fight against it by the human immune system. This article uses the combination of TCM pharmacology and immunology theory to give examples of diagnosis and treatment, to explore the feasibility of modern TCM pharmacology theory in clinical treatment and hope to develop another kind of thinking in the future.

Keywords: Covid19; Huang Lian Jie Du Tang; Sha Shen Mai Dong Tang; Huo Xiang Zheng Qi San

Introduction

新冠疫情自 2020 年年初肆虐迄今已有两年余，从 Alpha 变种到 Omicron 变让症状也从危急重症转轻症，但感染率与传染率却较快速，带来的后遗症也给康复者带来生活上的不便，当中包括疲劳、气短、心悸、咳嗽、口干、脑雾等无法解释的症状。

在 2022 年在新马泰的患者感染新冠流行的 Omicron 变种后，常见的症状为咽喉疼痛、咽干咳嗽，以及疲劳、肌肉痠痛为主。而中医在治疗新冠病毒均在引经据典，从中药药理学研究进行开方治疗新冠病毒甚少，藉论文能起抛砖引玉之效，让临

床看诊开拓另一番新思路。

Methodology

1. Material

复方颗粒剂

1.1 黄连解毒汤 (Huang Lian Jie Du Tang)

产自台湾胜昌制药。

每克含有的有效成分：黄连、黄芩、黄柏、山栀子各 0.125g

1.2 藿香正气散 (Huo Xiang Zheng Qi San)

产自台湾胜昌制药

每克含有的有效成分：大腹皮、茯苓、白芷、紫苏叶、藿香、生姜各0.06g，陈皮0.04g，桔梗、半夏、白术各0.03g，炙甘草、大枣各0.02g

1.3沙参麦冬汤 (Sha Shen Mai Dong Tang)

产自台湾天明制药

每克含有的有效成分：沙参、麦冬各 0.25g，玉竹0.17g，冬桑叶、扁豆各0.13g，甘草0.08g

2. Method

所有调剂复方颗粒剂均以200ml热水冲泡，待温服用，一日二次，每次6g。

Cases

验案1

杨XX，女性，32岁

RTK自我检查阳性3日来电视讯问诊，症状为喉咙疼痛2日，声音嘶哑阵发性喘息，咽干咳嗽，疲乏，食欲低下，夜寐欠安易醒，大便正常，轻度发热，舌脉略。

处以科学中药5日，一日2次，每次6g，温水冲服。方子为黄连解毒汤 6g；藿香正气散 4g；沙参麦冬汤 3g。

隔日来电喉咙疼痛及声音嘶哑减轻，遵嘱继服。

5日后讯息通知RTK已转阴性，另处以百合固金汤6g及玉屏风散6g一周，一日2次，每次6g，温水冲服后来电诉诸症减轻。

验案2

李XX，女性，45岁

RTK自我检查阳性1日经友人推荐来约网诊，症状为气短喘息，咽干口渴，纳食欠佳，不欲饮食，夜寐烦躁不安，大便溏，否认发热，自测体温37.5°C，舌脉略。

处以科学中药5日，一日2次，每次6g，温水冲服。方子为黄连解毒汤 6g；藿香正气散 3g；沙参麦冬汤 4g。

药用3日后来诊，咽干口渴，夜寐烦躁不安等症状减缓，欲再开药解决气短喘息症状。

一周后随访，告知首诊5日后测RTK已转阴性。

验案3

林XX，男性，63岁

RTK自我检查阳2日来约网诊，症状为头痛，咽喉疼痛，伴喘息口渴，食欲低下，夜寐入睡困难，大便溏稀，自测体温38°C，舌脉略。

处以科学中药5日，一日2次，每次6g，温水冲服。方子为黄连解毒汤 6g；藿香正气散 3g；沙参麦冬汤 4g。

一周后致电跟进情况，回覆药用3日症状均缓解，待5日后测RTK。未随访。

Discussion

临床上中医办证论治的思维，进行开方治病，在新颖流行疾病面临一定的局限性。目前临床上对于病毒性感染尚未见特效药治疗，仅靠人体免疫系

统与之对抗是唯一的方法。而人体免疫系统如果过度激活，也存在可能造成细胞因子风暴(cytokine storm)，进而造成多个脏腑组织损伤、全身炎症反应综合征，而威胁生命。

现代中药药理学研究思路不只是应用现代科技提取其化学成分，而是从经典古籍的方剂中，溯本求源找出方中各个中药的正条品，经过筛选确定其药效及有效成分的研究也在生物医学的发展下逐渐打开迷思。

使用方剂：

1. 黄连解毒汤 (Huang Lian Jie Du Tang)

在古籍《外台秘要》记载用以清热解毒的方剂。在现代中药药理学的研究中发现其在抗病毒作用可抑制病毒 mRNA 表达水平亦减少炎症反应，也能抑制病毒RNA复制。研究发现黄连解毒汤所含成分汉黄芩素能够减轻炎性细胞的肺组织浸润，抑制肿瘤坏死因子- α (TNF- α)、白细胞介素-1 β (IL-1 β)、白细胞介素-6(IL-6) 和白细胞介素-10(IL-10) 产生，进而改善肺部损伤起到保护作用[1-3]。

2. 藿香正气散 (Huo Xiang Zheng Qi Shan)

出自《太平惠民和剂局方》，主要作为解表化湿、理气和中功效。在中药药理学的研究中，藿香正气散具有调节免疫功能，能显著改善脾脏、胸腺功能、降低血清白细胞介素-6(IL-6) 的含量，增加血清免疫球蛋白G 含量，提升免疫功能 [4]。同时能促进 T 淋巴细胞上的抗原蛋白质 CD4 和 CD8 的表达，抑制肿瘤坏死因子- α (TNF- α) 的产生，并调节促炎 / 抗炎因子水平 [5]。

3. 沙参麦冬汤 (Sha Shen Mai Dong Tang)

方在《温病条辨》中记载，功效为清养肺胃，生津润燥。在现代研究中发现可增强机体的免疫功能，在常规西药治疗的基础上加服沙参麦冬汤可提高肺炎患者的临床疗效[6]。

感染新冠病毒后会激活免疫细胞，释放IL-6和其它炎症细胞因子，而过多的 IL-6 会导致患者体内出细胞因子风暴，但其对肺部组织的修复和重塑对患者痊愈发挥一定的作用。应用中药药理学理论，结合应用黄连解毒汤、藿香正气散和沙参麦冬汤在治疗新冠病毒确诊者也不失为一个好方法。

黄连解毒汤在患者感染新冠病毒后，起到抑制病毒 mRNA 表达及复制同时也能减少炎症反应，对肺部起到减轻炎性细胞的组织浸润，起到护肺作用；藿香正气散在治疗的角色扮演着辅佐的角色，同时再配合沙参麦冬汤来调控免疫功能。

Conclusion

综合以上，探讨从中药药理学的角度挖掘出俱临床疗效的中医药，用以治疗难治之疑难杂证，以逆转急转而下病情发展，不失为另一个可行性的治疗思路。

在应对未来未知的疾病，不只是传染病，各类疾病可行性的思考方向，从机体作用机转、路迳等

分子生物学甚至是免疫学的角度结合，探讨中药单方或复方萃取有效成分介入进而达到治疗效果。

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The Herbal Medicine of *Vernonia cinerea* Tea Comparing the Effectiveness of Herbal Medicine with Placebo to Anti-cigarette Smoking U-Thong Hospital Suphanburi, THAILAND.

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ABSTRACT

Introduction: *Vernonia cinerea* is a herb in ASTERACEAE family, that is packed in the National list of essential Medicines (NLEM) and anti-appetized food when before eat. Smoking is cessation rates increased among those who received medications and counseling and recommendations, and research has been taken from Thai healer to make anti-smoking drugs.

Objectives: The aim are study to :1) To trial test of Herb *Vernonia cinerea*. 2) Research on herb placebo. 3) study to side effects herbal medicine .

Methodology: Using are evaluated an interview form. And statistical success program statistics, This study for two group the experimental study of double bilnd .

The results : The average number of smoking The average number of cigarettes smoked was 8 cigarettes per day after 10 days of attendance. The number of cigarettes smoked 6-7 cigarettes per day after 15 days of participation in the trial group was statistically significantly reduced ($P < 0.05$) because this study also impaired the control of variables.

Conclusion: The study this of *Vernonia cinerea* tea , This herb contain nitrates and nitride, It causes nausea and vomiting. It cause to minimum the cigarret , so it was the result of making cigarette to decreased pills, and sometime many used to make anti-appetite medications. Thun auther to having a food before eating will also cause you to lose weight too.

Keywords : *effectiveness , Vernonia cinerea tea , herbal medicine of placebo , Suphanburi , anti - cigarret smoking*

Introduction

According to the Ministry of Public Health [1,2], (Health Policy for all 12 provinces in Suphan province is one of 12 provinces to achieve this policy by defining 37 basic necessities. For public health work The non-smoking campaign is a fundamental necessity , article 33, which aims to make people aged 14 and over to be at least non-smoking. 90% of the 1993 survey found that U-Thong has 12% of the buried addicts , and the GOP and the U-Thong Hospital have herbal and production work to reduce smoking and are distributed throughout all districts of Suphanburi province and 2 interested parties. Over last to make 8 reliable amulets, thus conducting comparative battles. Effectiveness of Ya -

Mho – Noi tea [3,4] with herbs to comparative A placebo to reduce smoking, followed up every 5 days until 15 days in a total of 73 participants , was the source of research on the effectiveness of white flower grass and herbs. The total time of 15 days for trial.

Thais have known how to use herbs since the Sukhothai era, and it goes back a long way. To the era of King Chaivoraman VIII [8] in those days, the territory was extended to the border. On the Eye of Phraya or Aranyaprathet, came to sweep the colonies, and then he had a physical disease, leprosy, and there was evidence of drug use during World War II. The medicines that are still used today have been used by the Royal Medicine

Book of Chanthaburi (Department public health of Chanthaburi) [4], and in the days before Ayutthaya used herbal medicines to treat diseases in Thailand. Herbs, traditional medicine. Research and development of medicinal drugs from herbs, but the 5th Economic and Social Development Plan 1985 began clinical research for the use of turmeric herbs [5] . Thieves, mushroom chums, sweet potatoes, phayayo, and aloe vera. In the hospital. A type of herb that is packaged in many Thai medicines and has been used since ancient times, which is considered an ancient clinical trial, white flower grass. Scientifically named *Vernonia cinerea*, folk medicine [6,7] has found that white flower grass is taken to treat diseases such as lowering blood pressure. Asthma Treatment Hepatitis Treatment Reduce fever, joint pain, diuretics. The treatment of gallstones includes lower cigarette cravings, which are currently used for white-flowered grass herbs to stop smoking because they are local herbs that are widely used in Thailand. Easy to find and cheap. Studies that used white-flowered tea for smoking cessation, but found that most pulpits were difficult to eat and somewhat cumbersome, which white-flowered grass still has in its form. Capsules make it more convenient to eat.

Therefore, the World Health Organization has given priority and support for smoking control or Smoking cessation continues, with the "World no tobacco day" held on May 31st of every year to jerk off. Countries are aware of the dangers and losses caused by smoking, so Thailand has organized smoking control activities towards the goal. "Non-smoking Thai society"1 Herbal white flower grass [7] . Alternatives to reduce cravings for cigarettes Thailand's Smoking Situation According to the 2015 Report on Smoking In Thailand, the Thai population is 15 years of age or older. Up to 10.9 million smokers (19.58 %) found male smoking rates equal to 39.16% gender. Females were 1.76% considering smoking of the Thai population aged 15 and over. The trend is that the Thai population aged 15 and over who consumed tobacco in 1991 was 12.26 million (32.00%), a decrease to 10.9 million. In 2015, there was an average decrease of 0.5% and the proportion of smokers was found to be 95.18% in 1991 and gradually decreased to 85.88% of smokers. It was found that tobacco consumption rates. Thailand has a steady decline, and therefore there is not much disparity during the year, so it is necessary to have measures in place. As the tobacco industry currently has a new marketing strategy, it may result in an increase in smoking trials each year and secondhand smoke exposure is still observed in various public places, with increased rates of illness and death from tobacco-related diseases. Smoking is the cause of NCDs, which are diseases related to the habits or behaviors of life that we create ourselves. Smoking is the leading cause of the world's population's premature death or premature death. Severe and long-lasting disabilities due to smoking are the leading cause of many acute and

resilient diseases per male. And those closest to you who are exposed to tobacco smoke, such as vascular diseases. Heart disease, pulmonary emphysema, cancer in organs and smoking are also the leading causes of pain and death. In 2008, an estimated 5 million people worldwide died from smoking and 8 million were expected to increase annually by 2030. Of these, 9.5 million smokers were regular smokers and 1 million smokers were crowbars, compared to a 2006 survey that showed a slight decrease in the number of smokers while here.

The Office for National Statistics [6]survey found that nearly 11 million Thais over the age of 15 still smoke. Of these, 9.5 million smokers were regular smokers and 1 million smoking remnants, compared to a 2006 survey that showed a slight decrease in the number of smokers at the time. [8,9] Having a strong and determinedness towards smoking cessation [10] can lead to smoking cessation. However, smoking cessation rates in groups that are consulted and recommended only. Only 7% were found after following. 1 year and smoking cessation rate increases in the group receiving medications and counseling and recommendations, which are approximately less common. [12] . Smoking is the leading cause of the world's population's premature death or premature death [13]. Severe and long-lasting disabilities due to smoking are the leading cause of the disease. Acute and abundant male-related. And those closest to you who are exposed to tobacco smoke, such as vascular diseases. Heart disease, pulmonary emphysema. Organized cancer and smoking [] are also the leading causes of preventable pain and death. According to statistical data [14], in 2008, an estimated 5 million people worldwide [15] died from smoking , and 8 million are expected to increase annually by 2030.

Methodology

This study the experimental study of double blind - randomize controlled study are evaluated using an interview form. In the trial group, results were found to be used during the 5th, 10th and 15th days by drug users and interviewees. It is not known which project participants (Double Blind Case control - study) reduce bias 1)This research model is experiment research , it is an experimental study. Compare by giving the experiment group It is divided into two groups, the total number of which is the group that uses white-flowered grass herbal medicine as an experimental group, and the group that uses placebo herbs as a control group.The results are evaluated using an interview form. Results of herbal medicine during 5, 10 and 15 days

- 1.) Case - Control Study is an experimental, blind (Double- blind)
- 2.) Practical definitions used in the research of white flower grass herbs

The experimental group consisted of 3.72 white flower grass and 0.71 grams of chrysanthemum and pandan leaves, for a total of 5 grams.

- Control group. The herbal medicines to brew are chrysanthemums and mandanus leaves, 25 grams each, for a total of 5 grams.
- Experimental group, tea makers, herbal medicine, white flowers, The envelope includes: 1) Mho -Noi -grass number 3.57 and 2) Chrysanthem amount 0.71 g 3) Pandanus leaves 0.71 g each

How to use brew white flower herb in 1 sachet per glass of water (or if you are working outdoors, you can make herbal medicine into 3 sachets of water cylinder. Per bottle of water, Bottle of fish sauce 750 cc.) to eat after meals.

Result

The average number of smoking sessions is 13 – 19 cigarettes per day. Average pumping time 14 – 21 cigarettes per day after 5 days of attendance. The average number of cigarettes smoked was 8 cigarettes per day after 10 days of attendance. The number of cigarettes smoked 6-7 cigarettes per day after 15 days participation in the trial group was statistically significantly reduced ($P < 0.05$) because this study also impaired the control of variables.



Figure 1 *Chrysanthemum morifolium*, *Vernonia cinerea*, and *Pandanus amaryllifolius* Roxb.

Table 1 Shows the average percentage of cigarettes smoked decreased, comparing real drugs and placebos.

Estimate date	xperimental drugs	Number of people	\bar{X}	SD	MEAN	P-value	2-tail Sig.
5 Days	Real drugs.	41	56.348	28.290	4.418	0.117	0.579
	Placebo	32	60.1979	34.403	6.082		
10 Day	Real drugs.	41	70.7201	28.230	4.409	0.641	0.758
	Placebo	32	72.8646	30.831	5.450		
15 Day	Real drugs.	41	70.4152	32.303	5.045	0.279	0.259
	Placebo	32	78.5156	27.199	4.808		

Side effects of herbal medicine: That could be caused by drinking a brew. They are may experience dry throat, untested mouth, ignorance of taste, or dizziness and nausea. When smoking continues, some people, tongue tea, loss of appetite. Therefore, do not eat before eating or have nausea when taking medication, some people have a smell of cigarettes. I don't want to keep smoking.

A comparison of the lower percentage of smoking cigarettes in the toddler group and control group showed that the percentage of cigarettes smoked decreased significantly from the control group because P -value = 0.05 by the control group had an average percentage of cigarettes smoked more than the trial group. As shown in Figure 1

Table 2 Shows the volunteer to separate of herbs On 15 days.

Results	Number (Individual)	percent
No reduction	4	5
reduce	45	62
Quit smoking	24	33

The reason most smokers voluntarily reach 9 percent (65%) In this group, there were 39 cases of smoking (60%) and 24 quit smoking (33%), with only 4 cases (5%). The second reason for wanting to reduce costs has 45 cases (62%) in this group can reduce smoking.

Symptoms of not wanting to smoke cigarette in the trial group It found that 15 cases were 37 percent. The control group found that 47 percent of smoky stinks were present in the trial group. Found 17% anorexia In the trial group, 17 cases were 42%, the control group found 11 cases, 34%. Mouth numbness, numb tongue, loss of appetite. In the 17 trials, 42% of the controlling group, 11 percent, 34 percent. Nausea When I took the pills in the trial group, Eight cases were found, 20%, the control group found, only 10 cases, 37% of whom saw similar side effects. It may be said that before starting the project, it has been indicated that the symptoms may be experienced as a result of the participants possibly feeling it themselves, as shown in table.

They could be caused by drinking a brew. They may experience dry throat, unsalted mouth, ignorance of taste, or dizziness and nausea. When smoking continues, some people, tongue tea, loss of appetite. Therefore, do not eat before eating or have nausea when taking medication, some people have a smell of cigarettes. I don't want to keep smoking.

Table 3 Shows general information about who in the project

Reason / population participant	Male (N)	Female (N)
1. Population	41	32
2. Smoking period (year+- SD)	20.54 +- 12.69	20.06 +- 14.32
3. Average age (years+- SD)	40.10 +- 15.70	42.59+- 16.45
4. Number of cigarettes smoked (molly/day +- SD)	-	-
- Before joining the program	19.29 +- 13.82	21.66 +- 18.23
05 days after participation	8.37 +- 8.01	6.72 +- 18.22
10 days after participation	5.59 +- 7.57	4.94 +- 7.95
15 days after participation	5.67 +- 7.89	3.97 +- 7.48

Table 4 Shows the reasons for participation of project

Reason	N	Trial group (N of Anti smoke)	N	Control group (N of Anti smoke)
1. Voluntary	39	13	26	9
2. Others recommend	4	1	1	0
3. Forced	14	5	10	5
4. Have a medical condition	15	6	20	8
5. People around you are disgusted	7	20	8	2
6. Fear is the cause of the disease	22	14	15	9
7. Want to reduce costs	22	13	20	8

Discussion

There should be more control over the variables, but there are too few samples. According to this study, it can allow participants to reduce and quit smoking. Down by 62 per cent and 32 percent respectively.

Conclusion

A total of are 73 in this study in the program to reduce cigarettes, the majority of which showed that more men than females had an average age of 40 to 45 years. The average number of smoking sessions is 13 – 19 cigarettes per day. Average pumping time 14 -21 cigarettes per day after 5 days of attendance. The average number of cigarettes smoked was 8 cigarettes per day after 10 days of attendance. The number of cigarettes smoked 6-7 cigarettes per day after 15 days participation in the trial group was statistically

significantly reduced ($P < 0.05$) because this study also impaired the control of variables.

Side effects of herbal medicine: That could be caused by drinking a brew. They may experience dry throat, unsalted mouth, ignorance of taste, or dizziness and nausea. When smoking continues, some people, tongue tea, loss of appetite. Therefore, do not eat before eating or have nausea when taking medication, some people have a smell of cigarettes. I don't want to keep smoking.

Competing Interests

No

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Author contributions

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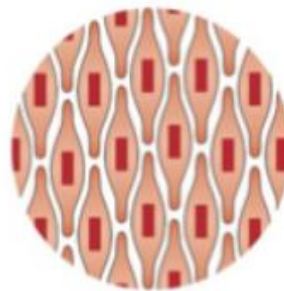


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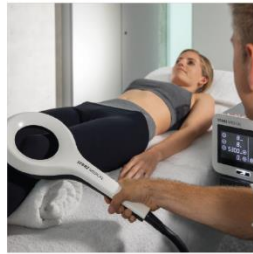
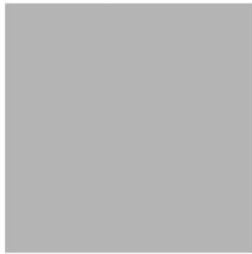
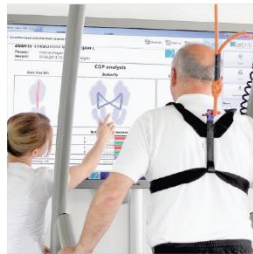
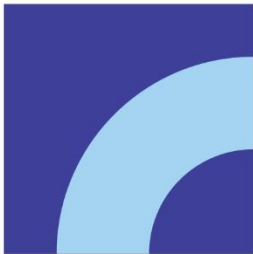
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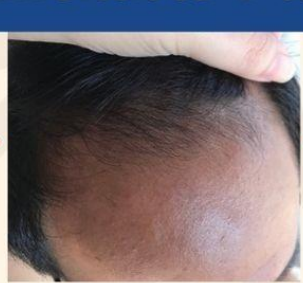
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