The 40th Annual Scientific Meeting
the Physiological Society of Thailand
INTERNATIONAL CONFERENCE

"Integrative Physiology, Medicine, Food and Technology:

Building a Healthy Future"

Venue: Pullman Khon Kaen Raja Orchid Hotel

Date: May 2-4, 2011

Hosted by:

- Department of Physiology, Faculty of Medicine, Khon Kaen University

Jointly organized by:

- The National Research Council of Thailand
- Nursing Department, Srinakarind Hospital, Faculty of Medicine, KKU
- Faculty of Associated Medical Sciences, Faculty of Nursing, Faculty of Pharmaceutical Sciences, Faculty of Dentistry, Faculty of Veterinary Medicine, and Faculty of Agriculture, KKU
- North-Eastern Neuroscience Association, Thailand
- Cardiovascular Research Group, KKU
- Exercise Sciences and Health Promotion Research Group, Faculty of Medicine, KKU
- Infertility and Fertility Sciences Research Group, Faculty of Medicine, KKU

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Vol. 24, No. 1 (Suppl), April-September 2011

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A welcome message from the PST President

I am delighted to welcome you to Khon Kaen for the 40th Annual Meeting of the Physiological Society of Thailand, the International Conference "Integrative Physiology, Medicine, Food and Technology: Building a Healthy Future", May 2-4, 2011.

This international meeting is gracefully organized by the Faculty of Medicine, Khon Khan University, in collaboration with the Physiological Society of Thailand and the National Research Council of Thailand. The conference integrates various scientific sessions of great interest delivered by several internationally and nationally renowned speakers. In addition, it also features 4 distinguished lectures, 6 symposia, 6 workshops, and over 70 oral and poster communications.

I do hope that all dear speakers and participants would have a chance to enjoy state-of-the art knowledge and exchanging experience, and also to strengthen our friendship.

With outstanding educational and social programs, I would like to convey my sincere appreciation to the organizing committee who has worked very hard to make this conference a joyful and memorable experience.

With my best wishes and personal regards,

Supatra Lohsiriwat

Associate Professor Supatra Lohsiriwat MD, FRCMT
President, the Physiological Society of Thailand

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A welcome message from the Conference Committee

It is with great pleasure to welcome you to the 40th Annual Meeting of the Physiological Society of Thailand International Conference held from 2 - 4 May, 2011.

This conference aims to provide fascinating insights, the most up-to-date multidisciplinary scientific knowledge, and practical know-how on a wide range of topics where scientists and medical professionals can meet and exchange ideas and information. The scientific programs also contain various workshops to enhance research and teaching. A commercial exhibition will also be organized alongside the conference and will feature the latest innovations and technology in the related fields.

Through this event, I hope that the participants will have a good chance to work together to open a new medical paradigm, which will integrate basic physiology, complementary medicine and advanced technology in order to build a healthy future. Participants and their accompanying persons will also enjoy the famous warmth and hospitality of Thais in various social events. We are confident that your stay in our lovely city of Khon Kaen will be a memorable experience.

Jintanaporn Wattanatorn
Conference Chair

Upa Kukongviriyapun

Wilaiwan Khrisanapant
Scientific Committee

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Scientific Program

Monday May 2, 2011
Venue: Orchid Ballroom 1-2

0730-0830 **Registration**

0830-0915 **Welcome & Opening Remarks**

Associate Professor Supatra Lohsiriwat, PST President
Assistant Professor Jintanaporn Wattanathorn, Conference Chair
Professor Soottiporn Chittmittrapap, NRCT Secretariat

0845-0915 Flying with the elephant : role of NRCT for the leveraging of the National research system

Professor Soottiporn Chittmittrapap, NRCT Secretariat

0915-1015 **Distinguished Lecture 1: Ouay Ketusingh Memorial Lecture**

Cardiovascular biomechanics: from physical theory through engineering practice, to patient diagnosis

Professor Stephen E. Greenwald
Pathology Group, Blizard Institute of Cell & Molecular Science,
Barts & The London School of Medicine & Dentistry, Queen Mary University of London, London, UK

1015-1030 Exhibition/Refreshment

1030-1200 **Symposium 1**
Inflammation, stress, immunity, diabetes and metabolic disease

1030-1100 **S1-1**
Stress and immunity - Reactivation of lymphocryptovirus monitors status of stresses

Professor Takafumi Ishida
Human Biology & Genetics, Department of Biological Sciences, Graduate School of Science, University of Tokyo, Japan

1100-1130 **S1-2**
Von Willebrand factor in cardiovascular disease

Associate Professor Nantarat Komanasin
Department of Clinical Microscopy, Faculty of Associated Medical Sciences, Cardiovascular Research Group, Khon Kaen University, Khon Kaen, Thailand

S1-3
1130-1200 The antineoplastic effects of nerve growth factor in mouse tumor growth

Assistant Professor Shingo Takatori
Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,Okayama University, Japan

1200-1300 **Lunch/Noon Symposium** (Sanofi-Aventis Thailand Ltd.)

How to manage acute coronary syndrome and stroke case in 24 hours

Professor Piyamitr Sritara
Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
Dr. Phongsak Intharaphet
Queen Sirikit Northeast Heart Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
Dr. Vichai Senthong
Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

1300-1430 **Symposium 2**

New approaches and tools in cardiovascular diseases

1300-1330 **S2-1**
Methods for studying sympathetic neurotransmission in the vasculature

Associate Professor William R. Dunn
School of Biomedical Sciences, University of Nottingham Medical centre, Queen's Medical Centre, Nottingham, UK

1330-1400 **S2-2**
Hyperglycemia and hyperinsulinemia induce neurogenic vascular dysfunction in type I and II diabetic model rats

Assistant Professor Yoshito Zamami
Department of Molecular Design for Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

S2-3
Endothelial ion channels: emerging therapeutic target

1400-1430

Assistant Professor Wattana B. Watanapa
Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

1430-1515 **Oral presentations**
Chair: Professor Pawinee Piyachaturawat
Co-chair: Associate Professor Yuvadee Wongkrajang

1430-1445 **O-01**
Biodegradable nanocomposites for tissue adhesive

Anirut Raksujarit, Kritsada Boonchom, Sittiporn Punyanitya

1445-1500 **O-02**
Mechanical properties of sintered submicrometer hydroxyapatite-bioglass composite

Sittiporn Punyanitya, Sakdiphon Thiansem, and Anirut Raksujarit

1500-1515 **O-03**
Effects of banana (*Musa sapientum* L.) supplemented diet on spatial learning and memory in male rats

Varoth Lilascharoen, Sukanya Jaroenporn, Jirarach Kitana, Noppadon Kitana

1515-1545 **Poster presentations [P-01 *to* P-30]/Refreshment**

1545-1700 **Oral presentations (cont.)**
Chair: Professor Pawinee Piyachaturawat
Co-chair: Associate Professor Yuvadee Wongkrajang

1545-1600 **O-04**
Mulberry fruit improves memory impairment and enhances cholinergic function in vascular dementia

Pratchaya Kaewkaen, Terdthai Tong-Un, Jintanaporn Wattanathorn, Supaporn Muchimapura, Suphachai Tiyavorranun, Wiroje Kaewrueng, Sataporn Wongcharoenwanakit

1600-1615 **O-05**
Wound healing activity of *Anacardium occidentale* in streptozotocin-induced diabetic rats

Cholathip Thipkaew, Jintanaporn Wattanathorn, Panakaporn Wannanon, Supaporn Muchimapura, Wipawee Boongvang, Panee Sirisa-ard, Surapol Nawathakarnkijkul, Sunee Chadrasakow, Chatchai Wattanapiromsakul

1615-1630 **O-06**
Liver x receptors regulate human organic anion transporter 1 in renal proximal tubular cells

Suticha Kittayaruksakul, Sunhapas Soodvilai, Varanuj Chatsudthipong

1630-1645 **O-07**
Neuroprotective and cognitive enhancing effects of *Anacardium occidentale* leaf extract

Wipawee Boongvang, Jintanaporn Wattanathorn, Supaporn Muchimapura, Panee Sirisa-ard , Surapol Nawathakarnkijkul, Sunee Chadrasakow, Chatchai Wattanapiromsakul

1645-1700 **O-08**
Chitooligosaccharide ameliorates inflammatory bowel disease through inhibition of NF-κ

Mohammad Yousef, Rath Pichyangkura, Varanuj Chatsudthipong, Chatchai Muanprasat

1700-1800 **PST Minutes of Meeting**

1830 - **Welcome Reception**

Tuesday May 3, 2011
Venue: Orchid Ballroom 1-2

0800-0830 **Registration**

0830-0945 **Distinguished Lecture 2: Dithi Chungcharoen Memorial Lecture**

Impact of functional foods on neural regulation and neurovascular protection

Professor Hiromu Kawasaki
Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan

0945-1200 **Symposium 3**
Functional foods and natural products for health promotion and disease prevention

0945-1030 **S3-1**
Functional foods for health promotion and disease prevention

Professor Ian A. Macdonald
School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK

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1030-1100 **Exhibition/Refreshment**

1100-1200 **Symposium 3 (Cont.)**

1100-1140 **S3-2**
Global health threat of cadmium: prevention through plant-based food components

Professor Soisungwan Satarug
Centre for Kidney Disease Research, University of Queensland School of Medicine, Princess Alexandra Hospital, Woolloongabba, Brisbane, Australia

1140-1200 **S3-3**
Role of phytochemical antioxidants in experimental models of oxidative stress-induced vascular dysfunction

Associate Professor Upa Kukongviriyapan
Department of Physiology, Cardiovascular Research Group, Faculty of Medicine, Khon Kaen University,Khon Kaen, Thailand

1200-1300 **Lunch/Noon Symposium** (MSD Co., Ltd. and Takeda Co., Ltd.)

Endometriosis and infertility

Associate Professor Kanok Seejorn
Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
Associate Professor Supat Sinawat
Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
Dr. Thitima Chaisrisawatsuk
Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
Dr. Yaowapa Chongpensuklert
Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

1300-1400 **Symposium 4**
Assisted conception: an integrative approach of reproductive sciences and medicine

1300-1320 **S4-1**
Assisted conception: milestones and clinical applications

Associate Professor Supat Sinawat
Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

1320-1400 **S4-2**
Assisted conception: present status and future applications

Associate Professor Teraporn Vutyavanich
Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

1400-1530 **Oral presentations**
Chair: Professor Nateetip Krishnamra
Co-chair: Assistant Professor Chucheep Praputpittaya

1400-1415 **O-09**
Liver X receptors agonists attenuate arginine vasopressin-induced chloride secretion in MDCK-C7 cells

Promporn Raksaseri, Sunhapas Soodvilai, Varanuj Chatsudthipong

1415-1430 **O-10**
Tetrahydrocurcumin protects against cadmium-induced endothelial dysfunction in mice

Wanida Donpunha, Poungrat Pakdeechote, Patchareewan Pannangpetch, Veerapol Kukongviriyapan, Kwanjit Sompamit, Chada Phisalaphong, Upa Kukongviriyapan

1430-1445 **O-11**
Passive smoking and risk of cervical cancer in northeast Thai women

Sitakan Natphopsuk, Wannapa Settheetham-Ishida, Supat Sinawat, Chamsai Pientong, Pissamai Yuenyao

1445-1500 **O-12**
Effect of ginsenoside Re on whole-cell currents of human coronary artery endothelial cells

Suporn Sukrittanon, Wattana B Watanapa, Katesirin Ruamyod

1500-1515 **O-13**
Screening of the total phenolic compound, antioxidative properties and neuropharmacological activities of *Moringa oleifera* Lam. leaves extract

Woranan Kirisattayakul, Terdthai Tong-Un, Jintanaporn Wattanathorn, Supaporn Muchimapura, Panakaporn Wannanon

1515-1530 **O-14**
Effect of phytoestrogen from *Curcuma comosa* Roxb. on Wnt/β

Kanit Bhukhai, Kanoknetr Suksen, Pawinee Piyachaturawat, Apichart Suksamrarn, Arthit Chairoungdua

1530-1600 **Poster presentations [P-31 *to* P-58]/Refreshment**

1600-1700 **Symposium 5**
New approaches and tools in neuropsychological disorders

1600-1640 **S5-1**
Associate Professor Naiphinich Kotchabhakdi
Neuro- and Behavioural Biology Center, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakorn Pathom, Thailand

1640-1700 **S5-2**
Cancer stem cells in children brain tumors from bench research to bedside application

Dr. Wiyada Punjaruk
Department of Physiology, Faculty of Medicine, Khon Kaen University, Thailand

Wednesday May 4, 2011
Venue: Orchid Ballroom 1-2

0730-0830 **Registration**

0830-0930 **Distinguished Lecture 3**

Complementary and alternative care: bridging bench and bedside

Associate Professor Lorna K.P. Suen
The Nethersole School of Nursing, The Chinese University of Hong Kong SAR

0930-1000 **Distinguished Lecture 4**

From bench to bedside with advanced light microendoscopy

Associate Professor Wibool Piyawattanametha
National Electronics and Computer Technology Center (NECTEC)
& Faculty of Medicine Chulalongkorn University (MED CU)

1000-1020 **Exhibition/Refreshment**

1020-1200 **Symposium 6**
Exercise, lifestyle modification, nutrition and meditation

1020-1050 **S6-1**
Dimensions of CAM in exercise, lifestyle modification, nutrition and meditation

Professor Alex Hankey
Institute of Ayurveda and Integrative Medicine, SVYASA, Prashanti Kutiram, Jigani, Bangalore District, Karnataka, India

1050-1120 **S6-2**
Possible role of mild enjoyable exercise for improving cognitive functions in the elderly

Professor Hideaki Soya
Laboratory of Exercise Biochemistry & Neuroendocrinology, University of Tsukuba, Graduate School of Comprehensive Human Sciences, Tennoudai, Tsukuba, Japan

1120-1140 **S6-3**
Yoga and holistic health

Associate Professor P. Nazini
Department of Food Science, Periyar University, Salem-11, Tamilnadu, India

1140-1200 **S6-4**
Physical activity and exercise in obese individuals

Associate Professor Wilaiwan Khrisanapant
Department of Physiology, Exercise Sciences and Health Promotion Research Group, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

1200-1300 **Lunch/Noon Symposium** (Sri Supphaluck Orchid Co., Ltd.)

Cashewy juice: a miracle drink

Assistant Professor Jantanee Uriyapongson
Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand
Assistant Professor Jintanaporn Wattanathorn
Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
Assistant Professor Nareumon Leelayuwat
Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

1300-1430
Workshop 1: Aromatherapy and food elements
Workshop 2: Thai massage
Workshop 3: Mind-body exercise: Yoga, Ruesidadton, Chi Kung
Workshop 4: Music therapy
Workshop 5: Tools to enhance research and teaching
Workshop 6: Transcranial magnetic stimulation

1430-1500 **Refreshment**

1500-1630 **Workshop 1-6 (Cont.)**

1630-1700 **Awards Presentation and Closing Remarks**

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Distinguished Lecture 1: Ouay Ketusingh Memorial Lecture
Monday May 2, 2011

Professor Ouay Ketusingh (1908-1990)
MD, Honorary PhD in Medicine, Diploma-Chemiker, Dr.rer.nat.

Education

1932	Doctor of Medicine, Chulalongkorn University
1935	Honorary PhD in Medicine, Chulalongkorn University
1936	Diploma in Tropical Medicine, University of Hamburg
1939	Doctor of Sciences & Diplom-Chemiker, Dr.rer.nat

Work

1933-1943	Lecturer, Siriraj Hospital School of Medicine, University of Medicine
1946	Head of Physiology, Siriraj Hospital School of Medicine, University of Medicine
1952	Professor of Physiology, Siriraj Hospital School of Medicine, University of Medicine
1965	Vice Rector of University of Medicine
	Head of Pharmacology, Siriraj Hospital School of Medicine, University of Medicine
1968	Retired
	Director of the Sports Science Center, Organization of Sport Promotion of Thailand

Distinguished Positions

The first Dean of the Faculty of Pharmacy, University of Medicine (1940-1943)
Expert Consultant to the Khon Kaen University Council
President of the Council on Thai Sports Medicine
Head of Thai-Indian Cultural Center
Honorary Research Committee, Federation of German Sports Medicine
President of the Federation of Asian Sports Medicine
President of the Charity for the Promotion of Thai Traditional Medicine
Director of Ayuravej Wittayalai (Shewakomalpat)
Member of the National Legislative Assembly
Senator
Member of the Medical Council

Highest Accolades

Mahawachilalongkorn
Das krase Faredeenkreust (Federation of Germany)

Research and publications

He had published several articles in the fields of physiology, biology, sports medicine and biomedical electronics.

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Distinguished Lecture 1: Ouay Ketusingh Memorial Lecture
Monday May 2, 2011

Professor Stephen E. Greenwald
Institute of Cell and Molecular Science, Pathology Group,
Queen Mary University of London, United Kingdom
Email: s. e.greenwald@qmul.ac.uk

Following a BA in Natural Science (Chemistry) at Hertford College Oxford (1971) and a PhD in Medicine (Pathology) from Guy's Hospital Medical School (1975), Steve Greenwald obtained a British Heart Foundation Junior Research Fellowship working on arterial elasticity in children. This was followed by a position as research assistant in the Pathology Department of The London Hospital Medical College investigating the relationship between arterial structure and mechanical properties and how this relationship is modified by vascular disease. He has been on the academic staff of The Medical College since 1980 and obtained a chair in Cardiovascular Mechanics in 2001. Since 2002 he has been head of intercalated degrees and an Associate Director of The Interdisciplinary Research Centre in Biomedical Materials at Queen Mary University of London. He was elected as the Vice President of the International Society of Pathophysiology in 2006.

His current research interests include a search for mechanical factors in the genesis of arterial disease, with emphasis on the role of fatigue failure in arterial elastin, foetal programming of essential hypertension and wave propagation in arteries. The link between the elastic properties of arteries, pulse pressure and the mechanical load on the heart is now thought to be the explanation, at least in part, for the widely recognised association between raised vascular stiffness and an increased risk of cardiovascular morbidity and mortality. On the applied side, the interest in arterial mechanical properties has lead to studies evaluating the efficacy of compliant intravascular stents and the development of a novel optical method for the non-invasive measurement of arterial compliance. The possibility of applying similar optical techniques to the measurement of cardiac output and the assessment of endothelial function is now under active investigation.

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Distinguished Lecture

Cardiovascular biomechanics: from physical theory through engineering practice, to patient diagnosis

Stephen E. Greenwald

Abstract

Cardiovascular biomechanics can be defined as the study of the interaction between the physical properties of the heart and the arteries; and the physical laws which govern the time-varying pressure and flow of the blood flowing through them. Its aim is to answer at least two related questions. Firstly, if the physical properties of arteries change due to ageing and or disease, how will this affect blood flow through them? Secondly, do these changes in pressure and flow lead to remodelling of the heart and arteries and what are the mechanisms underlying the remodelling. This article briefly describes a biomechanical approach to understanding the pathogenesis of vascular disease and outlines the way in which simple physical theory has been used to inform the development of a novel method for the measurement of arterial function, suitable for use in the developing and developed worlds.

Introduction

What is cardiovascular mechanics?

If nature had perfected the wheel, age-related isolated systolic hypertension and arteriosclerosis would not be a problem. The heart could have evolved as a rotary pump (or, more probably, as two rotary pumps in series, separated by the lungs) and the vascular system would function perfectly well, even if blood vessels were rigid. In reality, of course, the heart consists of two compound reciprocating pumps in series, each of which ejects an equal volume of blood, either into the pulmonary artery from the right heart or the aorta from the left. In order to accommodate this volume of blood without requiring an excessively high pressure, the aorta and pulmonary arteries must be able to expand and to store this volume for long enough to allow it to drain into the distal parts of the circulation and be ready to receive the volume ejected from the ventricles during the following heart beat. Most cardiovascular diseases (CVD) are associated with changes in the stiffness and dimensions of blood vessels and with the complex flow of blood within them and there is increasing evidence that mechanical factors are of major importance in the pathogenesis of these diseases,[1, 2] as well as during the 'normal' process of aging[3]. Given the high incidence of CVD and the resulting expense, it is therefore of interest and importance to the physiologist, the physician and the surgeon to understand the contribution of these factors.

Cardiovascular mechanics can be defined as the study of the interaction between the physical properties of the heart and the arteries; and the physical laws which govern the time-varying pressure and flow of the blood flowing through them. Its aim is to answer at least two related questions. Firstly, if the physical properties of arteries change due to ageing and or disease, how will this affect blood flow through them? Secondly, do these changes in pressure and flow lead to remodelling of the heart and arteries and what are the mechanisms underlying the remodelling. The possible synergy between these questions immediately suggests a third, namely: when considering the mechanical factors associated with the pathogenesis and development of CVD, which comes first? Does disturbed blood flow lead to changes in the mechanical properties of the heart and blood vessels, or do changes in these properties lead to disturbed flow? As one might expect, the two occur together and each can enhance the pathological effects of the other[3].

We have known for almost 200 years that pulse pressure is affected by the distensibility of the arterial system, its dimensions and the presence of wave reflections,[4]. During the last two decades there has been a steady increase in reports of an association between changes in arterial stiffness/raised pulse pressure and the development of CVD. Indeed there is now good evidence that increased stiffness and or pulse pressure may predict the onset and development of CVD at a stage before vascular lesions or external symptoms become evident[5-8]. Aortic stiffness is now used in estimating CV risk scores[6, 9, 10] and the prognostic value of such measurements is widely accepted. Therefore, there has been renewed interest amongst clinicians, engineers and materials scientists in existing and novel methods of measuring arterial elasticity and in developing new ways of diagnosing and treating CVD. An example from our own work on the application of physical principles governing blood flow and arterial stiffness will be outlined below.

Physical theory

Elasticity. *The heart and arteries are complex multi-component structures undergoing complex bending and twisting motion. To describe their behaviour fully and rigorously requires a theory that takes into account all the observed properties. These include:*

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- Anisotropy, (different properties in different directions)

- Nonlinearity, (material gets stiffer as it is stretched more)

- Viscoelasticity, (material gets stiffer the faster it is stretched)

Inelasticity, (material has different properties when stretched and when relaxed)

For the vascular physiologist, pathologist or clinician, a description in which blood vessel stiffness may be related to structural factors is required, so that useful diagnostic and predictive measurements can be made non-invasively and routinely. A simplified model of arterial elasticity has been used in many studies in which measurements are confined to the circumferential direction (that is the force required to increase the diameter or circumference, elastic non-linearity is treated by confining measurements to a relatively small pressure range, for instance between diastolic and systolic pressures; viscoelasticity is dealt with by assuming a constant heart rate during a relatively short measurement period and inelasticity is averaged out over the cardiac cycle. In this way a single number can be used to define how much force (or pressure) is required to increase the diameter of a blood vessel by a known amount and therefore this single number provides an index of functional stiffness. This functional stiffness depends on the stiffness of the materials which make up the arterial wall (its material stiffness) together with the thickness of the wall as a fraction of the vessel's radius. Thus, of two vessels made of the same material and having the same lumen radius, but different wall thickness, the one with the thicker wall will be functionally stiffer.

Haemodynamics

As the left ventricle beats it ejects about a cupful of blood into the aorta at a speed between 0.5 and 1 metre per second. The patterns of flow are complex and change not only in time, during the cardiac cycle, but also in space as the blood encounters curves and junctions throughout the vascular system. Furthermore, the flow may become turbulent when it emerges from a stenosis or stagnant when passing through an aneurysm. These complex normal and pathological flows can be measured with ultrasound or MRI and they can also be simulated numerically. Both approaches are necessary to understand the interaction between the flowing blood and the vessel wall, and may help in the design of more effective devices and treatments such as vascular grafts and stents.

When the left ventricle contracts it produces a wave of pressure which causes the aorta to expand to accommodate the volume of blood ejected. At the end of systole the pressure in the ventricle falls below that of the aorta and, as blood starts to flow back into the heart, the aortic valve shuts. During systole the combined effect of the contracting ventricle causes the wave of pressure to travel down the aorta (and up the carotid arteries) towards the periphery. This wave manifests itself as a ripple in the wall of the arteries it traverses and the speed that the ripple propagates depends on the stiffness of the arteries: the greater the stiffness, the higher the speed. (During diastole the energy stored in the stretched aorta continues to drive the blood downstream.) When the pulse pressure wave encounters a change in the properties of the arterial wall such as an occlusive lesion or a junction, like all waves it undergoes a reflection and these reflections travel back towards the heart. When a reflected wave encounters the wave generated by the next heart beat, the two can add together, producing a local increase in pulse pressure. Where this occurs depends on where the reflection is located and how fast the original and reflected waves travel which, as explained above, depends on how stiff the artery is. In many cases the original and reflected waves meet in the proximal aorta during systole in which case the heart is subjected to increased afterload tending to cause ventricular hypertrophy which, in turn can predispose to angina or exacerbate pre-existing ischaemic disease due to atherosclerosis. These effects are summarised in figure 1. The synergistic interaction between arterial stiffness, blood flow and pressure is summarised in figure 2. This approach based on simple physical principles allows one to investigate and understand the role of mechanical factors in the pathogenesis of cardiovascular disease. It also suggests new therapies based on treating the causes of vascular remodelling leading to altered stiffness,[11-13]. Furthermore it encourages the exploitation of simple physical principles in the development of non-invasive methods for diagnosis of arterial disease. In what follows I shall, as an example, outline the development of a novel method for the measurement of arterial stiffness and touch upon some of the problems associated with producing a device suitable for clinical use.

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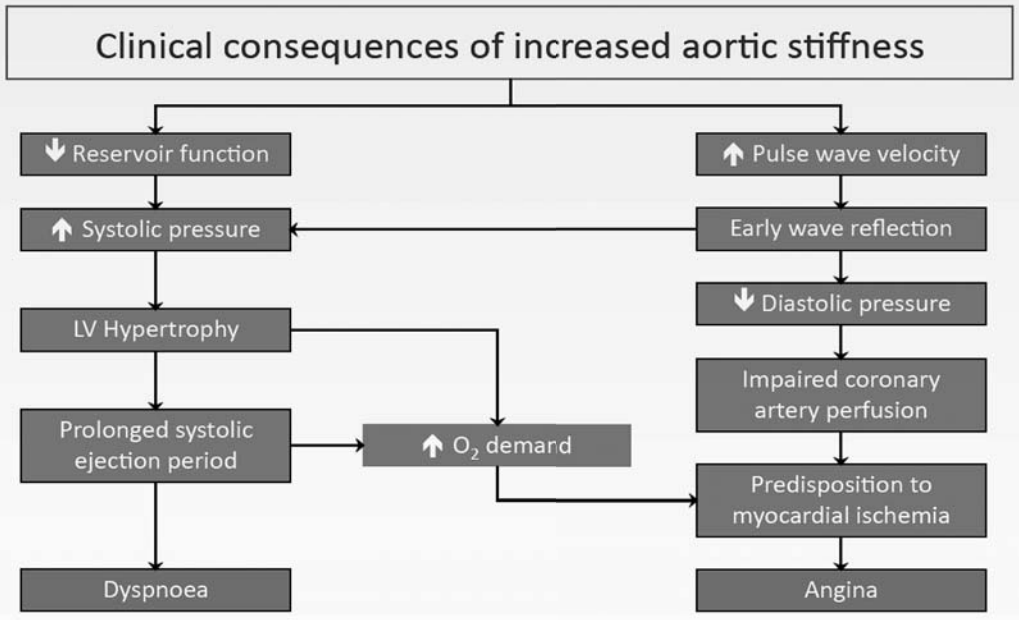

Figure 1. Increased aortic stiffness leads to increased cardiac load for two reasons. Firstly the myocytes must generate a greater tension to eject the required stroke volume into the aorta thus raising systolic blood pressure. This leads to cardiac remodelling dyspnoea and increased oxygen demand, as shown on the left of the diagram. Secondly the increased stiffness results in raised pulse wave velocity so that the reflected wave returns during systole thus raising systolic pressure yet further, as shown on the right. Both effects predispose towards angina. (Adapted from [1]).

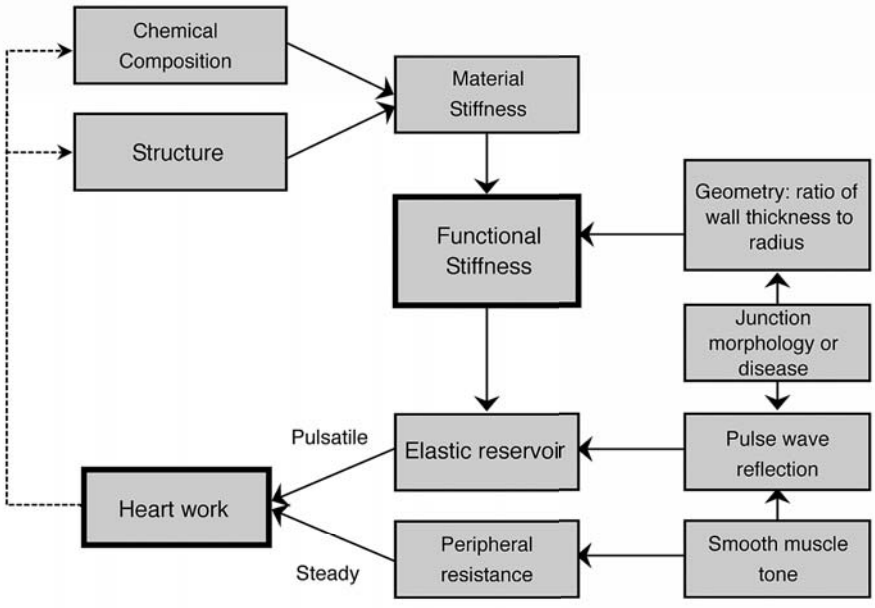

Figure 2. Interrelationship between vessel composition, structure, elasticity, geometry, elastic reservoir function and cardiac work. Functional stiffness, which determines pulse pressure and therefore the peak load on the heart, depends on the combined effects of composition and geometry. Hypertrophic arteries which have a greater functional stiffness even if their material properties are unaltered, become stiffer and therefore increase this load as the heart attempts to maintain normal levels of flow. Age related increases in stiffness have a similar effect. The dotted line represents a feedback loop indicating that changes in pulse pressure and flow can lead to remodeling, which in turn lead to further changes in pressure and flow. For instance raised mean pressure leads to medial hypertrophy, increased functional stiffness and PWV. This results in augmented pulse pressure and further remodeling of the heart and conduit arteries. Redrawn from [16].

From theory to practice

Development and testing of a prototype measuring system

As described above, arterial stiffness may be measured by determining the speed of the pulse wave generated by the heart. To do this it is necessary to detect the pulse at two sites a known distance apart and then to measure the time it takes for the pulse to travel between them. There are several commercial methods in current use for doing this non-invasively, including tonometry for detecting the pressure

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wave, duplex or echo tracking ultrasound, or MRI for detecting the resulting change in arterial diameter and Doppler ultrasound for detecting the transient flow wave as blood flows away from the centre of the vessel in response to the travelling pressure wave. These commercial systems are expensive and, in general are not suitable for routine or screening use. During the last few years we have developed a novel system for detecting the pulse based on the principle of *photoplethysmography* (PPG) and have shown that with the probes placed over the carotid and femoral arteries it is able to estimate aortic PWV with reasonable accuracy and repeatability [14].

None of these methods can detect the pulse in the aorta itself, relying on pulse detection in the carotid and femoral arteries, with possible uncertainty in the distance measurements and timing [10]. All these methods require skilled operators and a high level of expertise to hold the probes and get adequate signals. Furthermore, the femoral approach, requiring exposure of the skin near the groin is not patient friendly. We have adapted a method described in a US patent based on the idea of aortic pulse detection via the intercostal arteries [15], which solves many of the aforementioned difficulties. However there appear to be no literature reports of its development. The principle, as realised in our approach, is to detect the pulse in the skin of the back fed by the intercostal arteries which, being close to the skin can be easily visualised by PPG. It is assumed that because the intercostal arteries are close to the aorta, the arrival of the pulse accurately reflects the timing of the aortic pulse wave. Figure 3 shows how this is realised.

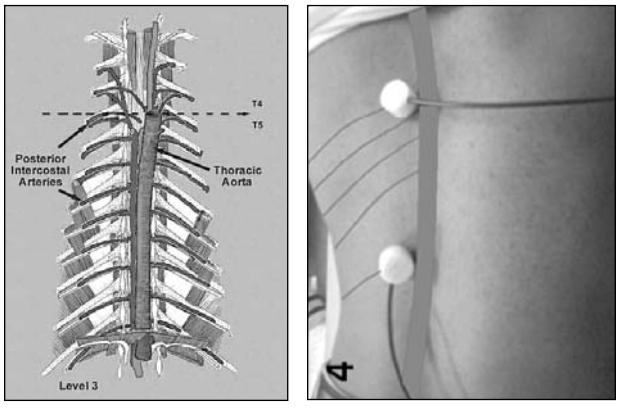

Figure 3. Left hand panel, anatomy of the ribs and intercostal arteries showing their proximity to the aorta (From http://www.wesnorman.com/thoraxlesson5.htm). Right hand panel, measurement of aortic PWV via the intercostal arteries showing the two PPG probes. The thick red line represents the position of the aorta, ventral to the spine and the thin red lines mark out the intercostal spaces.

Before developing this idea, it is necessary to provide good evidence that the timing assumption is justified. In other words the physical/mechanical principles behind the device's mode of operation must be understood. To do this we are investigating the propagation of light in a physical model of the chest in which the optical properties of the aorta, the intercostal arteries, the muscles of the back the sub-dermal capillaries and the skin are simulated. At the same time we are carrying out a computer simulation of these processes. At each stage the experimental and computer modelling results will compared and differences used to fine-tune both models in such a way as to minimise the differences between them.

Is there a need?

However strong the scientific evidence behind a new diagnostic device, before it can be accepted by the medical profession (which is, rightly, conservative) it must be thoroughly validated to show that the results it produces compare closely to an accepted *gold standard*. Typically a preliminary validation study on a small number of healthy subjects is performed in which the results obtained with the new device are compared to a device or technique which is widely accepted and has been assessed in many independent studies. For pulse wave velocity measurements the gold standard is intra-arterial measurement of pressure at two sites, which being invasive and requiring angiography to position the pressure measuring device(s) is ethically unacceptable in healthy subjects. In the development of our PPG system we have therefore approached the validation in two stages. Firstly on healthy volunteers we have compared PWV measurements obtained with Doppler ultrasound in which the pulse is detected in the carotid and femoral arteries, with our PPG method in which the probes are positioned over the intercostal spaces. Doppler ultrasound is a well established technique although, as mentioned above, it does not allow direct measurement of aortic pulse wave velocity. Nevertheless the agreement in PWV between the two approaches was satisfactory. The second step in the validation process is currently in progress. It involves PWV measurements obtained as in step 1, from the intercostal spaces and comparison with intra-arterial PWV values from patients undergoing elective coronary angiography in whom the pressure measuring catheters are already in place. Ethical permission must, of course, be obtained for measurements on all subjects whether healthy or hospital in-patients. For these experiments this has been relatively straightforward because our new device is non-invasive and the invasive catheter measurements are part of the routine diagnostic procedure undertaken by our patient group.

Our device, in common with many others resulting from investigations based on biomechanical principles, may be suitable for development into a system suitable for routine clinical use. Before investing time, energy and money in this development it is necessary to consider the following questions:

- Is there a clinical need?
  - Do the clinicians perceive the need or is it likely they can be convinced of it?
  - Do the healthcare planners perceive the need or can they too be convinced?
- Is the technology feasible?
  - What alternatives already exist and how does the new device improve on these?
  - Will it be cost effective?

These questions are not always easily answerable and can not necessarily be answered until large scale (and therefore expensive) validation studies are carried out. It is therefore necessary, having achieved a successful *proof of principle*, to seek more substantial funding, either from a government agency or from industry or from a combination of the two. In the UK and Europe such sources of combined funding are accessible, but further discussion of funding is beyond the scope of this brief account. An equally serious hurdle to investigating the effectiveness of a device which may have commercial potential, is approval from the *Medical and Healthcare Products Regulatory Agency*. This is a governmental organisation, similar to the FDA in the USA, whose function is to ensure that all medical devices or drugs meet stringent safety standards as laid down by the European Union. To gain this approval it is necessary to demonstrate in the laboratory that the device is electrically safe, poses no radiation hazard (whether electromagnetic or ionising), will not interfere with the proper functioning of other devices in the vicinity and can be sterilised if necessary. Furthermore a detailed risk analysis must be performed to assess the likelihood and consequences of any part of the system's failing. This includes the effects of errors in any computer software on which its proper functioning depends. Although these requirements can appear daunting and the testing can add significantly to the development costs, they ensure that the design of the device and the way it is used clinically is properly scrutinised at an early stage before it is too late to modify.

Finally, if the device has been validated, if it has been shown to be safe and to produce reliable and useful clinical data, it must then be transformed into a user friendly system, "packaged" and marketed. If they have not already gained funding from a commercial company many practicing scientists with entrepreneurial skills choose to establish their own spin out companies, often with the support of their university to produce a commercial prototype. For those who may not wish to follow this path, there is the possibility of selling or licensing their technology to a medical device or pharmaceutical company.

To conclude, cardiovascular biomechanics is a subject that has, in the past, drawn together mathematicians, physicists, physiologists and engineers, motivated by the wish to understand that most fascinating of nature's machines – the human organism. Today, as in all other types of biomedical research, the scientist has joined forces with the technologist and entrepreneur. This combination of skills is essential not just for 'high-tech high cost' medical innovations, affordable only in the developed world but also for simpler solutions for use in developing countries as well, such as the device described here. Cardiovascular Biomechanics has helped and will help realise the theme of this conference, "Building a Healthy Future".

Acknowledgement

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Distinguished Lecture 2: Dithi Chungcharoen Memorial Lecture
Tuesday May 3, 2011

Professor Dithi Chungcharoen (1917-1993)
MD, Honorary PhD in Medicine, PhD

Education

1938	Doctor of Medicine, Chulalongkorn University
1947	Honorary PhD in Medicine, Chulalongkorn University
1952	PhD, University of London, UK
1980	Honorary PhD in Sciences, Mahidol University

Work

1938	Lecturer in Department of Physiology, Siriraj Hospital School of Medicine
1961	Professor of Physiology, Siriraj Hospital School of Medicine
1966	Head of Physiology
1970-1978	Head of physiology, Siriraj Hospital School of Medicine
1978	Retired

Distinguished Positions

Head of the Medical Curriculum, Siriraj Hospital School of Medicine
Head of the Master Degree Curriculum, Siriraj Hospital School of Medicine
Head of the 2-year Medical Technology Project
The National Research Council, Medical Science Branch (1960)
President of the Thai Physiological Society

Highest Accolades

Mahawachilalongkorn

Research and Publications

He had widely published several articles in the fields of cardiovascular physiology, physiology of ageing and medical education.

Honorary Biography

Member of the Society of the Sigma XI Temple University Chapter (devoted to the Promotion of Research in Sciences)
Chaopayaprasadej Surentarathibodhi Award for the Outstanding Lecturer, Siriraj Hospital School of Medicine
Founder of Biomedical Electronics in Thailand
Founder of Workshops for maintenance of equipments for research and teaching
Innovation and modernization of instruments for physiology research
Founder of the Experimental Animal Housing, Siriraj Hospital School of Medicine
Founder of the integrative teaching between pre-clinical and clinical years

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Distinguished Lecture 2: Dithi Chungcharoen Memorial Lecture Tuesday May 3, 2011

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Hiromu Kawasaki, PhD, is a Professor of Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University.

Professor Kawasaki graduated from Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan (1969), and he earned the master and PhD degree from Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan (1971-1978). He was a postdoctoral fellow of Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, Illinois, USA (1980-1982).

In 1979, he became Associate Professor of Department of Pharmacology, Miyazaki Medical College, Miyazaki, Japan and moved to Okayama University in 1997 as an Associate Professor of Department of Pharmacy, Okayama University Hospital. In 1999, he became a full Professor of Department of Clinical Pharmaceutical Science, Faculty of Pharmaceutical Sciences, Okayama University, and in 2004 he moved to the present position.

Professor Kawasaki's research interests and specialties are Cardiovascular and Autonomic Pharmacology, Pharmacotherapy, Cardiovascular Physiology, Blood vessel Physiology and Pharmacology, Neuroscience of perivascular nerves. His research has been funded by a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Science and Technology of Japan, the Smoking Research Foundation, Kobayashi Research Laboratories (Japan).

Professor Kawasaki has published a total 255 papers including Nature, Pro Nat Acd Sci, J Pharmacol Exp Ther, Br J Pharmacol, Circ Res, Eur J Pharmacol, J Pharmacol Sci. He is a member of American Heart Association High Blood Pressure Research Council (USA) and Society for Neuroscience (USA), member and trustee of Japanese Pharmacological Society and Japanese Society of Eucommia, member and councilor of Japanese Society of Hypertension, Japanese Society for Circulation Research, Japanese Association of Cardiovascular Pharmacology. He was the President of the 113th Kinki Branch Meeting of The Japanese Pharmacological Society (2008), the President of the 38th Annual Meeting of Japanese Society of Circulation Research (2009) and the secretary general of the 130th Annual Meeting of Japanese Pharmaceutical Society (2010),

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Impact of functional foods on neural regulation and neurovascular protection

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Abstract

Insulin resistance, which is a physiological condition where insulin becomes less effective at lowering blood glucose, is associated with overweight and obesity in metabolic syndrome. The present study was designed to investigate whether natural products including honey-bee products (Royal Jelly; RJ and Propolis) and *Eucommia ulmoides* Oliv. leaves extract (ELE) prevent insulin resistance (hyperinsulinemia) and altered neural vascular regulation in fructose-drinking rat (FDR; experimental insulin resistance model) and Otsuka Long-Evans Tokushima Fatty rat (OLETF-R) (spontaneous insulin resistance model). FDR was produced by receiving 15% fructose solution in drinking water for 8 weeks using male Wistar rats (6 week-old). FDR had significant increases in plasma levels of insulin and triglyceride, an index of insulin resistance (HOMA-IR), and systolic blood pressure (SBP), but not blood glucose levels. OLETF-R at 14 week-old also had hyperinsulinemia with normal blood glucose level, increased HOMA-IR and hypertension. FDR and OLETF-R showed increase in adrenergic nerve-mediated vasoconstriction and adrenergic innervation and decrease in calcitonin gene-related peptide (CGRP) nerve-mediated vasodilation and CGRP nerve innervation. Oral treatment with RJ (100 and 300 mg/kg/day) or Propolis (Brazilian Propolis extract; 100 and 300 mg/kg/day) for 8 weeks significantly decreased plasma levels of insulin and triglyceride, HOMA-IR and tended to lower SBP. In OLETF-R, RJ or Propolis treatment for 4 weeks tended to decrease SBP and significantly reduced plasma insulin levels and HOMA-IR. In FDR, Oral treatment with ELE (500 and 1000 mg/kg/day for 4 weeks) significantly decreased plasma insulin levels and HOMA-IR and significantly lowered SBP. Furthermore, ELE treatment in FDR resulted in significant increase in CGRP-LI never density and significant decrease in TH-LI never density in mesenteric arteries of FDR. These results suggest that honeybee products and plant extract ELE could be an effective functional food to prevent insulin resistance associated with the development of hypertension.

Introduction

Insulin resistance, which is a physiological condition where insulin becomes less effective at lowering blood glucose, is associated with overweight and obesity in metabolic syndrome. When insulin resistance exists, more insulin is secreted by the pancreas to compensate insulin action, resulting in hyperinsulinemia. If this compensatory increase does not occur, blood glucose concentrations increase and Type 2 diabetes mellitus occurs. Thus, insulin resistance often progresses to full Type 2 diabetes mellitus. This is often seen when hyperglycemia develops after taking appropriate dieting such as high fat and high calorie diets, when pancreatic β-cells are unable to produce sufficient insulin to maintain normal blood sugar levels (euglycemia) in the face of insulin resistance. The inability of the β-cells to produce sufficient insulin in a condition of hyperglycemia transits from insulin resistance to Type 2 diabetes mellitus. Therefore, it is very important to prevent development of insulin resistance to reduce a risk for developing Type 2 diabetes mellitus.

Patients with Type 2 diabetes mellitus have high risk for complications of vascular diseases including atherosclerosis, angina pectoris and hypertension, and it has been suggested that there is a relationship between insulin levels and blood pressure.[1] Many clinical studies have shown that patients with Type 2 diabetes mellitus have frequently insulin resistance associated with hypertension, suggesting that insulin resistance and hyperinsulinemia contribute to the pathogenesis of hypertension.[2] In fact, insulin increases sympathetic activity[3] and renal sodium reuptake[2] and promotes proliferation of vascular smooth muscle cells,[4] which could increase the blood pressure. Moreover, hyperinsulinemia has been shown to contribute to increase sympathetic activity,[5] and a close association between insulin resistance and hyperinsulinemia has been suggested in essential hypertension.[6] This relationship implies that insulin resistance and hyperinsulinemia contribute to the pathogenesis of hypertension, and furthermore, that insulin might play an important role in malfunction of the cardiovascular system. On the other hand, some investigations have shown that insulin acts as an endogenous vasodilator.[7] Therefore, it is hypothesized that insulin resistance might induce hypertension due to decreased insulin-induced vasodilation and the imbalance between its pressor and depressor effects.[6] However, the association of insulin and blood pressure has remained controversial. Previously, we reported that in pithed rats without a central vasoreflex acute insulin infusion augments adrenergic nerve-mediated vasoconstriction,

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which is partially associated with inhibition of calcitonin gene-related peptide (CGRP)-containing vasodilator nerve (CGRPergic nerve) function as well as endothelium function.[8] This finding implies that hyperinsulinemia may elevate blood pressure by augmenting the sympathetic adrenergic activity.

Chronic administration of fructose to rats has been reported to cause insulin resistance, which is characterized by increased serum insulin and euglycemia.[9] Especially, a hyperinsulinemic state associated with hypertension was more prominently induced by fructose drinking than by fructose feeding.[10] Our previous studies demonstrated that chronic hyperinsulinemia in fructose-drinking rat (FDR), an experimental model for insulin resistance, increased adrenergic nerve-mediated vasoconstriction and decreased CGRPergic nerve-mediated vasodilation, resulting in abnormal neuronal regulation of vascular tone leading to hypertension.[11, 12] Therefore, it is expected that inhibition of insulin resistance prevents development of cardiovascular diseases such as hypertension.

We previously reported that chronic hyperinsulinemia and insulin resistance induced by fructose-drinking loading elicited hypertension associated with abnormal neuronal regulation of vascular tone in an *in vivo* study using pithed rats, which have no vasoreflex.[11] Recently, we reported that FDR showed significant increases in plasma levels of insulin, the glucose-insulin index, blood noradrenaline levels and systolic blood pressure (SBP), but not blood glucose levels, when compared with normal water-drinking rats (control rats).[13] In perfused mesenteric vascular beds of FDR, enhanced adrenergic nerve-mediated vasoconstriction and decreased calcitonin gene-related peptide (CGRP) ergic nerve-mediated vasodilation. [13] Furthermore, immunohistochemistry studies showed increased density of neuropeptide Y (NPY) immunopositive adrenergic fibers and reduced density of CGRP-immunopositive fibers in mesenteric arteries of FDR, suggesting that dysfunction of the neuronal vascular control system resulting from abnormal innervation of mesenteric perivascular nerves induced by the hyperinsulinemic state is responsible for the development of hypertension in FDR. [13]

Otsuka Long-Evans Tokushima Fatty rats (OLETF-R) (spontaneous insulin resistance and Type 2 diabetic animal model) in early stages at 8 to 25 weeks of ages increases body weight and develops spontaneously insulin resistance with hyperinsulinemia and euglycemia and elevates SBP. In late stages at more than 25 week-old, they showed decreased blood glucose levels (hypoglycemia) to develop Type 2 diabetes.

Insulin resistance silently develops because of no symptoms. Also, it is very difficult to diagnose as insulin resistance, since complicated clinical tests need. At preset, a very few drugs (thiazolisine derivatives) are available to treat insulin resistance. Therefore, if we could find natural foods and their ingredients, which have some preventive effects on development of insulin resistance, and take these functional foods as daily diets, it would be effective to prevent insulin resistance, which is considered to be a cause of various lifestyle-related diseases such as Type 2 diabetes mellitus and hypertension.. Thus, the present study was designed to investigate the effects of long-term administration of natural products including Royal Jelly (RJ) and Propolis and plant extract *Eucommia ulmoides* Oliv. leaves extract (ELE) on insulin resistance (hyperinsulinemia) using FDR[11] and OLETF-R.[14, 15]

Effects of RJ in FDR

RJ is a honey bee secretion that is used in the nutrition of larvae, as well as adult queens. RJ is secreted from the glands in the hypopharynx of worker bees, and fed to all larvae in the colony. RJ is known to contain three major nutrients including amino acids, vitamins and minerals.[16] Additionally, RJ has various biological activities such as a hypotensive effect and insulin-like action.[17, 18] Therefore, it is possible that RJ may have some effects on insulin resistance.

In FDR, which received 15% fructose solution in drinking water from 6 week-old to 14 week-old for 8 weeks and showed significant increases in plasma levels of insulin and triglyceride, Homeostasis Model Assessment ratio (HOMA-IR, an index of insulin resistance), and SBP, but not blood glucose levels, when compared with control rats. Oral treatment with RJ (100 and 300 mg/kg/day) for 8 weeks immediately after fructose loading significantly decreased the plasma levels of insulin and triglyceride, HOMA-IR, without affecting blood glucose or total cholesterol levels and tended to lower SBP. In isolated and perfused mesenteric vascular beds of FDR, RJ treatment resulted in a significant reduction in sympathetic nerve-mediated vasoconstrictor response to periarterial nerve stimulation and tended to increase the CGRPergic nerve-mediated vasodilator response to periarterial nerve stimulation, compared with those in untreated FDR. However, RJ treatment did not significantly affect noradrenaline-induced vasoconstriction or CGRP-induced vasodilation. These results suggest that RJ could be an effective functional food to prevent insulin resistance associated with the development of hypertension. [19]

Effect of RJ in OLETF-R

OLETF-R at 14 weeks of age showed increase in plasma insulin levels and HOMA-IR, normal blood glucose, and high SBP. OLETF-R at 10 week-old was orally treated for 4 weeks with RJ (10, 30 and 300 mg/kg). RJ treatment tended to decrease SBP and significantly decreased serum insulin levels and HOMA-IR. In isolated and perfused mesenteric vascular beds of OLETF-R, RJ treatment resulted in significant reduction of sympathetic nerve-mediated vasoconstriction and augmentation of CGRPergic nerve-mediated vasodilation, compared with that in non-treated OLETF-R. However, RJ treatment did not significantly affect the noradrenaline-induced vasoconstriction and CGRP-induced vasodilation, suggesting that RJ could be an effective and functional food to prevent development of insulin resistance.[20]

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Effect of Propolis in FDR

Propolis is a resinous mixture that honeybees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is known to contain effective ingredients including flavonoids, vitamins and minerals.[21] Propolis has various biological activities such as an anti-bacterial activity, anti-inflammatory activity, hypotensive effect, insulin-like action, anti-tumor activity and antioxidant activity.[22, 23]

Brazilian Propolis extract at doses of 100 and 300 mg/kg/day was orraly administered for 8 weeks immediately after Male Wistar rats (6 week-old) received 15% fructose solution in drinking water for 8 weeks. Treatment with Propolis in FDR significantly decreased plasma level of insulin, HOMA-IR and body weight, plasma triglyceride levels without affecting blood glucose and total cholesterol level and tended to decrease SBP. In isolated and perfused mesenteric vascular beds of FDR, Propolis treatment resulted in significant reduction of sympathetic nerve-mediated vasoconstriction and tended to augment CGRPergic nerve-mediated vasodilation, compared with those in non-treated FDR. However, Propolis treatment did not significantly affect the noradrenaline-induced vasoconstriction and CGRP-induced vasodilation, suggesting that Propolis could be an effective and functional food to prevent development of insulin resistance and hypertension.[24]

Effect of Propolis in OLETF-R

10-week old OLETF-R were orally treated for 4 weeks with Propolis (100 and 300 mg/kg/day) or vehicle (Control). Propolis treatment significantly decreased the plasma levels of insulin and HOMA-IR without affecting blood glucose levels and tended to lower SBP, compared with Control. In isolated and perfused mesenteric vascular beds of OLETF-R, Propolis treatment resulted in a significant reduction in sympathetic nerve-mediated vasoconstriction and tended to augment CGRPergic nerve-mediated vasodilation, compared with those in vehicle-treated OLETF-R. However, Propolis treatment did not significantly affect noradrenaline-mediated vasoconstriction and CGRP-mediated vasodilation. These results suggest that Propolis could be an effective and functional food to prevent development of insulin resistance in spontaneous insulin resistance model.[25]

Effect of Eucommia ulmoides Oliv. leaves extract (ELE) in FDR

The leaf of *Eucommia ulmoides* Oliv is commonly used as a traditional Chinese medicine to treat hypertension.[26] Recently, several pharmacological studies have reported that leaf extract of *Eucommia ulmoides* Oliv (ELE) also exhibits effects such as anti-hypercholesterolemia, anti-fatty liver, anti-oxidative stress and anti-obesity.[27, 28] Lee *et al*. reported that powdered *Eucommia ulmoides* Oliv leaves and water extract improved hyperglycemia in streptozotocin-induced Type 1 diabetic rats.[29] Furthermore, Park *et al*. reported that water extract of *Eucommia ulmoides* Oliv leaves ameliorated hyperglycemia and hyperlipidemia in Type 2 diabetes by modulating the glucose and lipid metabolic enzyme activities.[30] Accordingly, we investigate whether ELE treatment prevents the development of insulin resistance in FDR and whether ELE treatment ameliorates the abnormal distribution of perivascular nerves in the isolated mesenteric vascular beds of FDR using immunohistochemical methods.

In FDR, ELE at doses of 500 and 1000 mg/kg/day were orally administered once daily for 4 weeks immediately after 15% fructose-drinking loading. A 4 weeks treatment of FDR with ELE significantly decreased plasma insulin levels and HOMA-IR without affecting blood glucose levels and significantly lowered SBP in FDR. In immunohistochemical study, FDR showed significantly greater density of tyrosine hydroxylase (TH)-like immunoreactivity (LI)-containing nerves and significantly lower density of CGRP-LI-containing nerves in mesenteric arteries of FDR than those in control. ELE treatment in FDR resulted in significant increase in CGRP-LI never fiber density and significant decrease in TH-LI never fiber density in mesenteric arteries of FDR. These results suggest that long-term ELE treatment effectively prevents insulin resistance development and ameliorates abnormal perivascular innervation in FDR.[31]

Conclusion

These results suggest that RJ and PPL of honeybee products and ELE of plant extract could be an effective functional food to prevent insulin resistance, which produces dysfunctional neural regulation of vascular tone associated with the development of hypertension.

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Distinguished Lecture 3
Wednesday May 4, 2011

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Lorna K.P. Suen (RN, RM, BN, MPH, PhD) is currently an Associate Professor at the Chinese University of Hong Kong. She received both her degrees in Bachelor of Nursing and Master of Public Health in the University of Sydney, Australia, while she obtained her PhD in Nursing from the Hong Kong Polytechnic University. Her specialization and research interests are Complementary and Alternative Medicine (CAM), auriculotherapy, critical care nursing and sleep studies. She has published many articles in nationally and internationally refereed journals, as well as book chapters. She was granted membership of the Golden Key National Honour Society of Australia in 1994 and of the Sigma Theta Tau International Honor Society of Nursing since 2003. Currently, she serves as a member of the editorial board of several journals, and is likewise a reviewer of various journals. She is also active in several community organizations in Hong Kong as a consultant.

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Distinguished Lecture

Complementary and alternative care: bridging bench and bedside

Lorna K.P. Suen

Complementary and Alternative Medicine (CAM) as a treatment modality is increasingly becoming acceptable to the general public as proven by its widespread use around the world. CAM involves interventions that focus on the body, mind, and spirit; in short, it offers a holistic approach to healthcare. Therefore, a basic tenet of many CAM therapies is holism, which is also fundamental to the nursing practice. Nurses adopt CAM therapies because these offer additional treatment options for patients and help promote patients' well-being. However, the lack of sufficient knowledge and training, institutional support, and a clear organizational policy related to the use of CAM by nurses, and limited scientific evidence to support its usage have been reported as the main barriers to the utilization of CAM. In this light, recommendations will be made in order to "bridge the bench and bedside" for complementary and alternative care. Advanced Nursing Practice should expand its scope to include practice in CAM beyond what may traditionally be viewed as nursing. The further development of continuing education opportunities and the strengthening of the nursing curricula that focus on alternative modalities should also be advanced in order to support this practice in clinical settings.

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Distinguished Lecture 4
Wednesday May 4, 2011

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From bench to bedside with advanced light microendoscopy

Wibool Piyawattanametha

Biomedical research truly needs new advances in imaging. Existing modalities of *in vivo* imaging, such as magnetic resonance imaging or ultrasound, lack the spatiotemporal resolution required to image the fundamental building block of living tissue. By contrast, existing high-resolution techniques for imaging cells and their sub-cellular features are technologies that are best suited for *in vitro* experiments in tissue slices. Yet, the ability to make direct connections between human pathological symptoms/behavior and the underlying cells and molecules responsible for such behavior requires *in vivo* techniques that can image cellular constituents. Our group research aim is to develop novel high-resolution optical endoscopes to satisfy unmet needs in the clinical environment. These differ from medical endoscopes, which are generally larger and designed to image macroscopic abnormalities. Most importantly, this novel optical endoscopic imaging might suggest new approaches to disease diagnosis and treatment. This talk will be focused on the development a novel and portable confocal imaging modality integrated with icroelectromechanical systems (MEMS) technology and their clinical imaging applications (translational research). Confocal microscopy is an attractive tool for three-dimensional (3-D) imaging due to its optical sectioning property. Conventional single-axis confocal (SAC) microscopes have a tradeoff between resolution, field of view, and objective lens size, since a high numerical aperture (NA) lens is needed for sufficient resolution, and a long focal length is needed for a large FOV and working distance. A dual-axes confocal (DAC) microscope architecture has been proposed utilizing two overlapping low NA beams, which effectively decouples these tradeoffs. The other important advantage is the ability to achieve a much superior optical sectioning compared to the SAC design. The microscopes are miniaturized into two form factors (5-mm and 10-mm diameter). The imaging demonstrations of the probes were on both *ex vivo* and *in vivo* from mice and human for cancer oncology and genetic research.

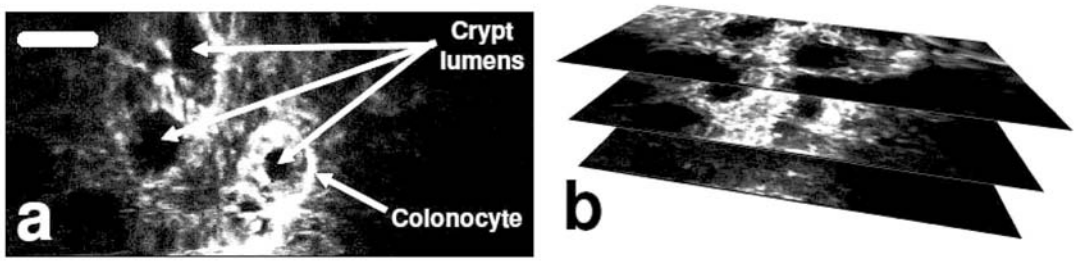

Figure 1. Ex vivo fluorescence images of normal human colon tissue (a and b). Rings of colonocytes and crypt lumens are clearly resolved. Freshly excised colon tissues are soaked in the Li-Cor IRDye 800 CW solution for 1 min before being irrigated with water to remove excess dye. All scale bars are 100 μm.

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Symposia

Symposium 1: Inflammation, stress, immunity, diabetes and metabolic disease

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Stress and immunity - reactivation of lymphocryptovirus monitors status of stresses

Takafumi Ishida

Gamma-lymphocryptovirus (LCV) is a group of herpes viruses that are commonly distributed in Old World simians (catarrhines) including humans. Each catarrhine species harbors its own LCV. It infects subjects in their early life and shows persistent infection probably for life. It infects latently showing usually no symptoms; however, recurrent reactivation of the virus occurs when the host's immune status declined. Physical and psychological stresses also can decline the immune status and reactivation of the dormant virus occurs. It is thus expected that LCV reactivation monitors status of the host's stresses. We studied LCV reactivation in the wild macaques serologically and in the chimpanzees molecularly in line with the presence of stressors.

Serological examination of LCV in the wild macaque groups (*Macaca fascicularis* and *Macaca fuscata*) suggested that physical and social stress might trigger the virus reactivation; the presence of antibodies to LCV early antigens was identified among pregnant females and the a-male in a population. Relationships between the rank of chimpanzees and chimpanzee LCV viral loads showed that tentatively higher rank individuals in the unsettled group shed more virus particles in blood circulation, probably because they were suffering from stresses to keep their rank status. As LCV particles are shed in the saliva, we can thus monitor LCV reactivation in the non-invasive mode. LCV reactivation, visualized either serologically or molecularly, would be a good tool for the monitoring stresses.

Keywords: *stress, viral reactivation, herpes virus, primates*

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The antineoplastic effects of nerve growth factor in mouse tumor growth

Shingo Takatori , Satoko Fukuhara, Mitsuhiro Goda, Narumi Hashikawa-Hobara, Yoshito Zamami , Hiromu Kawasaki

Our previous study has reported that nerve growth factor (NGF) suppresses the prostate tumor cell growth (*J Pharmacol Sci* 112, 463-466, 2010). Since NGF facilitates perivascular innervation, which plays an important role in regulation of vascular tone and blood flow, we hypothesized that neuronal regulation of tumor blood flow contributes to inhibition of tumor growth. The present study investigated possible mechanisms underlying NGF-mediated suppression of tumor growth. DU145 prostate carcinoma cells and HT1080 fibrosarcoma cells were subcutaneously implanted into nude mice. NGF or saline was infused using osmotic pumps or intermittent administration (s.c., once a day) for 2 weeks. To determine the direct effect of NGF on tumor cells, tumor cell proliferation using WST assay in vitro was examined. Density of vascular smooth muscle cells (VSM) and perivascular nerves in tumor vessels and tumor-induced angiogenesis were immunocytochemically detected. Tumor volumes of DU145 and HT1080 in saline group gradually increased, while tumor growth in NGF group with a continuous administration was markedly inhibited. However, the intermittent administration of NGF did not affect tumor growth. Significant suppressions of both tumor growths were continued even after withdrawal of NGF treatment. NGF prominently inhibited DU145 cells proliferation, but not HT1080 cells in concentration-dependent manner. VSM density of tumors in NGF group, but not microvessel density, was significantly greater than that in saline group. A distribution of perivascular nerves in tumor vessels was observed in NGF group, but not in saline group. These results suggest that NGF prevents tumor growth via the indirect effect, probably innervation or maturation of tumor neovasculature.

Keywords: *nerve growth factor, perivascular nerve innervations, tumor vessels maturation, reduced tumor growth*

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Von Willebrand factor in cardiovascular disease

Nantarat Komanasin

Von Willebrand factor (vWF) is a multimeric glycoprotein synthesised in endothelial cells and megakaryocytes. It is either secreted constitutively into plasma or alternatively stored in specific intracellular organelles known as Weibel-Palade bodies of endothelial cells or α-granules of platelets. Its plasma levels are determined by genetic factors including ABO blood group and vWF mutations/ polymorphisms, and by non-genetic factors including aging, impaired nitric oxide production, inflammation, oxidative stress and diabetes. Plasma vWF plays two essential roles in normal haemostasis. First, it mediates the interaction between platelets and damage endothelial cells of blood vessel wall. Second, it binds to procoagulant factor VIII, thereby serves as a carrier molecule for factor VIII. In addition to its role in normal haemostasis, vWF plays an important role in the pathogenesis of atherosclerosis and has been considered as a predictor of cardiovascular disease. An increase in plasma vWF levels has been proposed as a risk factor for adverse outcomes in the acute coronary syndrome. Although they are only weakly associated with the risk of coronary artery disease in the general population, they are a more promising risk factor for coronary artery disease in high-risk population including diabetes, elderly and individuals with previous cardiovascular events. Moreover, larger vWF multimers are more potent mediators of platelet thrombi formation and recent studies have shown that ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 repeats) synthesized predominantly by liver cells plays an important role in proteolytic regulation of high molecular weight vWF. Therefore, deficiency of ADAMTS13 leads to accumulation of ultralarge vWF multimers in the plasma results in the development of platelet-rich thrombi in the microcirculation. Thus, monitoring the changes of plasma vWF and ADAMTS13 antigen levels in the early phase might be valuable for predicting and preventing thrombosis during 1-year follow-up in patients with acute myocardial infarction. As mentioned above, both genetic and non-genetic factors determine the plasma levels of vWF, additional studies on the genetic determinants of both VWF levels and cardiovascular outcomes in the high-risk populations may help to monitor therapeutic and preventive interventions.

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Symposium 2: New approaches and tools in cardiovascular diseases

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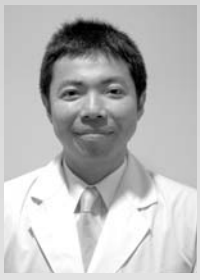

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A method for studying sympathetic neurotransmission in pressurized small arteries

William R. Dunn, Lakshman Goonetilleke, Poungrat Pakdeechote, Vera Ralevic

Small arteries (<400μm in diameter) are the most important determinants of vascular resistance and hence blood pressure. In vitro assessment of the function of these blood vessels is carried out using either isometric (wire myography) or isobaric (pressure myography) techniques. Surprisingly there are few reports examining sympathetic nerve mediated function in pressurized arteries. We have developed a method that allows small arteries to be attached between a suction electrode and a second cannula, linked to a servo-control pump, allowing vessels to be pressurized. A current is then passed between an electrode housed in the suction electrode and another on the outside of the vessel. With this technique we have shown that neurogenic responses in rat mesenteric small arteries increase in magnitude as the intraluminal pressure is increased, an effect associated with an increase in the functional role of ATP as a sympathetic neurotransmitter (Rummery *et* al, 2007). We have also examined the relationship between pressure and sympathetic function in small arteries isolated from normotensive (WKY) rats and spontaneously hypertensive rats (SHRs). Vasoconstrictor responses were larger in arteries isolated from SHRs compared to WKY rats, particularly at higher pressures (e.g. at 90mmHg, activation of the nerves with 10Hz stimulation (50 pulses, pulse width 1ms, stimulating voltage 10V) caused a vasoconstriction equivalent to a 34±2% reduction in diameter in arteries isolated from SHRs compared to a 10±1% reduction in diameter in arteries isolated from WKY rats (n=25)). This was associated with an increase in the magnitude of the P2X-receptor-mediated excitatory junctional potential (EJP) in SHRs (90mmHg; amplitude in vessels from SHRs, 10.8±1.2mV (n=6) compared to 7.3±1.2mV (n=5) in arteries from WKY rats, $p<0.05$). However, the vasoconstrictor response was effectively abolished by antagonists of either α_1-adrenoceptors or P2X receptors. Thus, vasoconstrictor responses are larger in arteries isolated from SHRs and, while there is evidence for an increased function of ATP in these vessels, the vasoconstrictor response involves a synergistic interplay between NA and ATP as sympathetic co-transmitters.

Keywords: *pressure myography, small arteries, ATP, noradrenaline. sympathetic*

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Hyperglycemia and hyperinsulinemia induce neurogenic vascular dysfunction in type I and II diabetic model rats

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Epidemiological studies have revealed that approximately forty percent of all patients with diabetes mellitus have complicating hypertension, suggesting that there is a relationship between hypertension and diabetes mellitus. In the present study, we investigated influence of chronic hyperglycemia and hyperinsulinemia on neurogenic vascular responses to spinal cord stimulation (SCS) and i.v. bolus injections of norepinephrine (NE) and calcitonin gene-related peptide (CGRP) in streptozotocin-induced diabetic rats (STZ; type I diabetes model) and fructose-drinking rat (FDR; experimental insulin resistance model).

In STZ (40 mg/kg i.v.) - induced diabetic rats showed severe hyperglycemia, low insulin concentration and normal blood pressure. In pithed STZ, adrenergic nerve-mediated pressor response to SCS was similar to that in normal rats. STZ-treated rats with increased BP showed greater CGRPergic nerve-mediated depressor response to SCS than that in normal rats. Male Wistar rats received 15% fructose as drinking solution for 10 weeks, which resulted in significant increases in plasma levels of insulin, glucose-insulin index and systolic blood pressure, but not blood glucose levels. In pithed FDR, CGRPergic nerve-mediated vasodilation was significantly decreased, while adrenergic nerve-mediated vasoconstriction was significantly increased, compared with control.

We conclude that hyperinsulinemia increases adrenergic vasoconstriction, which is partly associated with inhibition of CGRP nerve function, and that plasma insulin concentration may be responsible for altered neurogenic responsiveness and hypertension.

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Endothelial ion channels: emerging therapeutic target

Wattana B. Watanapa

The endothelium is a key player in cardiovascular control, as it is central to the regulation of vascular tone and blood flow. The synthesis and release of vasoactive substances, especially the vasodilator nitric oxide, from endothelial cells (ECs) are triggered by intracellular Ca^{2+} increase. Several types of ion channels in the EC surface membrane are pivotal in the regulation of Ca^{2+} influx. In EC, Ca^{2+} enters through non-voltage-gated Ca^{2+} channels, such as non-selective cation channel (NSC) and store-operated Ca^{2+} channels. The driving force for Ca^{2+} influx is determined by the resting membrane potential (RMP) set by various K^+ channels: inwardly rectifying K^+ channel (K_{ir}), small-, intermediate- and large-conductance calcium-activated K^+ channels (SK_{Ca}, IK_{Ca} and BK_{Ca}). Other channels in the EC include ATP-sensitive K^+ channel (K_{ATP}), Cl^- channel and stretch-activated cation channel (SAC). The contribution of each channel to the RMP, cytosolic Ca^{2+} levels and vasoactive compound release, at rest or during agonist stimulation, depends on the EC type (or location). Because early pathogenic change in common diseases, e.g. hypertension, coronary artery disease, cerebrovascular disease and atherosclerosis, involves endothelial dysfunction with defective vasodilator production, modulation of EC ion channels could provide an alternative preventive/therapeutic strategy in the treatment of these conditions. Studying endothelial ion channels as mediators of hormones and bioactive substances is another direction that has quickly become an active focus of research interest.

Keywords: *endothelium, ion channels, intracellular calcium, vasorelaxation*

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Symposium 3: Functional foods and natural products for health promotion and disease prevention

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Functional foods for health promotion and disease prevention

Ian A. Macdonald

Introduction

It is well established that consumption of a balanced diet is essential for the maintenance of good health. This has traditionally been focused around the macronutrients (fat, carbohydrate and protein) and micronutrients (vitamins, minerals and trace elements), together with fibre and water. This has given rise to the Recommended Daily Allowances (RDA) or Dietary Reference Values or Intakes (DRV/DRI) which are used in many countries as the basis of dietary recommendations to maintain the health of the general public Over the past few years there has been increasing interest throughout the world in potential health benefits of other components of foods and drinks, which have not traditionally been regarded as nutrients, but are now frequently referred to as 'functional foods'. Such components include the flavanoids (including green tea and cocoa extract), sterol esters, probiotics. There is also great interest in the potential health benefits of herbal preparations such as gingko biloba, but these preparations are outside the scope of the present review.

Functional Foods

The term 'functional foods' is somewhat misleading, as all foods which are normal constituents of the human diet fulfill a function of some type. For the purpose of the present review the term 'functional food' is taken to mean an effect of the food (or one of its ingredients) which is additional to the conventional nutritional properties of that food. Thus, a probiotic yoghurt or drink which is based on fermented milk is claimed to be a functional food not because of its contribution to calcium or protein intake, but because it is claimed to have an effect on intestinal bacteria which produces beneficial effects on gut function.

The regulatory authorities in different countries have different approaches to these functional foods, with some allowing any claims to be made regarding the health benefits which may be associated with consumption of the foods, whereas others require scientifically robust evidence of any claimed benefit. The latter approach is being taken in Europe by the European Food Safety Authority (EFSA), such that any health claim which a manufacturer wishes to make must be supported by a dossier of evidence which is reviewed and approved by EFSA. From a scientific perspective this approach is difficult to argue against as it will ensure that the public are not exposed to unsubstantiated claims about the potential health benefits of such functional foods. The following sections consider the potential health benefits which may be associated with some of the functional foods, but it must be emphasized that in most cases the evidence is not at the stage where a justifiable health claim could be made on the basis of solid scientific evidence.

1 – Sterol esters: there has been extensive interest in the effects of plant sterols on plasma cholesterol concentration for several decades. It is now clear that such compounds, when included in vegetable oil-based spreads or fermented dairy products have a small (approximately 10%) but consistent cholesterol lowering effect, with the effect appearing to be limited to LDL cholesterol. It is likely that such cholesterol lowering has beneficial effects on health by lowering the risk of cardiovascular disease, but this has not been tested in formalized long term clinical trials.

2 – Probiotics: there is substantial interest in the potential health benefits of consuming fermented dairy products containing strains of Lactobacillus and Bifidobacterium. These are referred to as probiotic foods, because they contain live bacteria, and a major effect of these foods is to alter the balance of the microbiota populations within the large intestine. Food products containing such bacteria have been available for several decades, especially in countries such as Japan and more recently throughout Europe. These food products have been claimed to improve gastro-intestinal health and general well-being, but there is little independent research in randomized, controlled trials to support such claims. However, there have been some recent studies using such probiotic products which have shown potential health benefits in relation to immune or allergic function, with a reduced incidence in upper respiratory tract infection in athletes during a northern European winter, and reduced severity of allergic rhinitis during the European summer. Intriguingly, the latter study only focused on cytokine responses and did not investigate whether symptoms were reduced. At present, EFSA has not produced any favorable opinions on the claimed health benefits of probiotic foods, and clearly more extensive data are needed from controlled human intervention studies before any conclusion can be drawn that such products have clear health benefits.

3 – Flavonoids: There has been interest since the early 1990's, in the potential beneficial effects of dietary flavonoids to delay the development of, and reduce the mortality from, coronary heart disease (CHD). Some of these studies used extensive dietary surveys to estimate total flavonoid intake from all sources, whilst other studies focused on specific dietary sources – usually tea, apples, or onions. In many studies, tea was the single largest contributor of measured flavonoids. It is worth noting that when these earlier studies were designed, it was not widely

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recognized that cocoa and other foods could be significant sources of flavonoids, and so potential contributions from these sources were overlooked. The Zutphen study showed a significant inverse relationship between total flavonoid intake and CHD mortality over a 5 year follow up period in elderly men. Major dietary sources of flavonoids (tea, onions, apples) showed trends for similar effects, but the study lacked the statistical power for effects of individual food sources to be significant. However, it is interesting to note that in this Dutch population, tea made a much more substantial contribution to total flavonoid intake than was seen subsequently in a study of US male health professionals.

Whilst the evidence for beneficial effects of dietary flavonoids, or specific sources such as tea, in the general population is thus a little contradictory, there is clearer evidence of benefit of tea consumption in those with CHD, or at very high risk of it. A case control study of 340 cases of acute myocardial infarction and matched controls, showed no differences in coffee or decaffeinated coffee consumption between the groups, but a significant relationship of tea consumption and reduced risk of myocardial infarction. A prospective study in Rotterdam showed beneficial effects of consuming 1-2 cups, or 4 cups, of tea / day compared to no tea consumption in lowering the risk of developing severe aortic atherosclerotic lesions. In another study of patients who had suffered a myocardial event, moderate tea drinking (median 2 cups/week) and heavy tea drinking (median 19 cups/week) were associated with 31 and 39% reductions in relative risk of death compared to no tea consumption.

Conclusions

There is now good evidence that regular consumption of phytosterol containing foods is associated with a reduction in plasma cholesterol, and extensive epidemiological evidence linking dietary flavonoids with reduction in risk of cardiovascular disease. However, controlled intervention studies are needed to establish whether foods rich in flavanoids confer a real benefit to health, and whether a person's background diet influences the effect observed. The potential health benefits of probiotic foods remain to be clearly established.

It is very important that as future claims are made about specific food products conferring health benefits, well designed appropriately controlled studies are undertaken to test such claims. Without clear scientific evidence to support any claimed health benefits of 'functional foods' the scientific community should remain skeptical and ensure that the public are not mislead by any spurious claims of beneficial effects.

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Global health threat of cadmium: prevention through plant-based food components

Soisungwan Satarug

Cadmium (Cd) is a non-essential metal, which is present in low levels in most soils, rocks and waters while high Cd amounts are associated with lead- and zinc-ores and rock phosphates, used for fertilizer production. Chronic exposure to low-level Cd, resulting in urinary Cd as low as 1μg/g urinary creatinine, has been shown to be a risk factor in the development of cancer in lung, breast, endometrium, liver, pancreas, kidney and urinary bladder along with a range of chronic diseases such as kidney dysfunction, osteoporosis, diabetes, hypertension, peripheral arterial disease, myocardial infarction, diminished lung function, periodontal disease, and macular disease, causing blindness. Relative to humans and animals, plants have greater capacity to tolerate Cd toxicity; no visible signs of toxicities in plants accumulating Cd to the levels toxic to humans. For over three decades, the sulphur-containing antioxidant α-lipoic acid has been used in the treatment of diabetic neuropathy. Now, α-lipoic acid, present in vegetables, notably spinach and broccoli, is a known mitochondrial protectant with promising metal chelation propensities. Efficacy of α-lipoic in Cd chelation and reversal of its toxicity remain to be explored along with its levels in indigenous Thai food plants for potential use in preventing Cd toxicity in high-risk individuals.

Food as a primary exposure source

Cadmium (Cd) is a non-essential metal which is present in low levels in most soils, rocks and waters while high amounts are associated with lead- and zinc-ores and rock phosphates, used for phosphate fertilizer production.[1] It is ranked number 7 in the priority list of 275 hazardous materials, indicating it is a prevalent contaminant at toxic wastes sites.[2] Cd is one of ten chemicals considered to be of major public health concerns.[3] It persists indefinitely in the environment, which facilitates the food-chain transfer and bioaccumulation.[4,5] Certain plants, (e.g. tobacco, peanuts, sunflower kernels, flaxseeds) and filter feeder animals (e.g. oysters and scallops) can accumulate high levels of Cd and are referred to as hyper-accumulators of Cd.[6,7] These lead to diet as a primary exposure source together with tobacco smoke.[1,8] Increasing presence of Cd in food crops has been attributed to its high rates of soil-to-plant transference in conjunction with extensive and excessive use of fertilizers, containing Cd.[9,10] In support of high rates of soil-to-plant transference, metal transporters responsible for Cd uptake has now been identified in rice and in the model hyper-accumulator plant, *Thlaspi caerulescens*.[11,12] In Sri Lanka, an outbreak of chronic kidney disease emerged after decades use of phosphate fertilizer for cultivation of high-fertilizer responsive rice varieties.[13]

Measured Cd content of various foods varied widely, depending on plant varieties, soil types, growing conditions and agricultural methods. Vegetables and cereals typically contain five times more Cd than fruit while the levels of Cd in kidney, oil seeds and cocoa beans were five times higher than in vegetables and cereals. Kidney and liver of animals contains relatively higher amounts of Cd than muscle (meat). Likewise, some crustaceans and mollusks accumulate large amounts of Cd. Relative to animal meat (muscle), crustaceans and mollusks contain relatively high Cd levels as such these food items should considered to be high-Cd food as with kidney, oil seed and cocoa beans. Dietary exposure assessment with Cd-in-food database suggested that staple food crops (rice, potatoes), vegetables, and cereal crops could be the most significant sources of dietary exposure. To a lesser degree, meat is a source of Cd in the diet. Typically, fish muscle, containing small quantities of Cd, does not tend to be a major source of Cd in the diet. Cd ingested in the diet appears readily bioavailable, evident from Cd levels detected in people without obvious exposure (non-smokers, and no workplace exposure). Further, studies in British Columbia, Canada and in the Torres Strait, Australia have shown elevated Cd body burden among those who habitually consumed high-Cd foods, notably oysters and offal.[14-17] Elevated body burden of Cd was noted among those who frequently consumed tofu.[18] Cd intake results in accumulation of Cd in all tissues and organs throughout the body, including kidney, liver, thyroid gland, pancreas, bone, heart, blood vessels (aorta), and eye tissue.[19] The most extensive accumulation occurs in the kidney cortex, followed by liver, pancreas and thyroid gland. Cd in the kidney and liver comprises one third of the total body burden of Cd. Urinary Cd levels correlate with kidney Cd content.[20, 21] Urinary Cd content has thus been used as a surrogate of kidney accumulation or long-term exposure (body burden).

Cigarette smoke as an additional exposure source

Inhalation of cigarette smoke through active and passive smoking provide a substantial Cd exposure source. This is because the propensity of the *Nicotiana* species to concentrate Cd independent of soil-Cd content. Cd tobacco content could be increased substantially when grown on soils high in Cd. This could underlie large variation in tobacco Cd content that appears to depend on the country of origin.[7, 22, 23] Cd content in six brands of cigarettes made in China varied between 1.3 and 2μg/g whereas it was 0.9

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and 1.2 μg/g in two other imported brands.[23] In a more recent analysis, Cd content of tobacco in Chinese cigarette brands varied from 2.0 to 5.4μg /g tobacco and these levels were higher than Canadian brands.7 An equivalent to 0.7 to 2.7 μg Cd per cigarette can thus be estimated, assuming each cigarette comprises 0.5 g tobacco. Cd content in mainstream smoke recorded in another study was between 0.025 and 0.035 μg/cigarette. Cd oxide, generated from burning of tobacco, is highly bioavailable-approximately 30-40% of the inhaled Cd oxide is absorbed into systemic blood circulation of smokers while another 10% is deposited in lung tissues. Smokers thus have elevated Cd uptake and accumulation in their tissues, organs, which result in higher Cd in blood and urine, compared to those who never smoke. Women of childbearing age in the United States had on-overage blood Cd of 0.40μg/L with smokers showing blood Cd of 0.88μg/L, which was 2-time high than overall average level and 3–4 times higher than did non-smokers (0.29μg/L). [24]

Global health threat of cadmium

Chronic intake of high-dose Cd causes Itai-itai disease, characterized by severe damage to the kidney and bone, resulting in multiple bone fractures due to generalized osteomalacia and osteoporosis.[25, 26] In 1989, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established the safe dietary intake guideline known as the Provisional Tolerable Weekly Intake (PTWI). The first PTWI for Cd was set as 400-500 μg per person per week.[27] It was subsequently adjusted to 7 μg per kg body weight per week (WHO 1993) and more recently to 25 μg per kg body weight per month.[28, 29] This guideline considered kidney as a prime toxicity target and it was recognized that these PTWI values represent a very modest safety margin between exposure in normal diet and exposure that produces deleterious kidney effects.[28] The current guideline for an intake rate of 25 μg per kg body weight per month translates to urinary Cd limit at 5.24μg/g creatinine at 50 years of age.[29] However, there is growing evidence that long-term intakes of dietary Cd, producing urinary Cd below the recommended exposure limit 5.24μg/g urinary creatinine, is associated with a range of organ-specific adverse health effects and distinct pathologies in a variety tissues and organs other than kidney and bone.[19, 30,31]

Reported adverse effects of long-term exposure to low-level Cd were chronic kidney disease, osteoporosis, diabetes, hypertension, peripheral artery disease, myocardial infarction, diminished lung function, periodontal disease, and macular degeneration. Of further concern, Cd exposures have been associated with increased risks of cancer of various sites such as lung, breast, endometrium, liver, pancreas, kidney and urinary bladder.19 In Belgium, mortality rate rose by 20% in low exposure area and it rose by 44% in a high exposure area, among those with a two-fold increase in Cd burden.[32] Mortality rose by 25% in a low-exposure area and it rose by 33% in a high-exposure area among those with a two-fold increase in blood Cd. Average blood Cd among cohort participants was < 2 μg/L, while average daily urinary Cd excretion was < 2 μg. In the U.S., a 4.29-fold increase in cancer mortality was noted among men as their urinary Cd level Cd rose from <0.21 μg/g creatinine to > 0.48 μg/g creatinine.[33] This male preferential effect was observed despite lower body burden in men (mean urinary Cd 0.28μg/g creatinine) than in women (mean urinary Cd 0.40 μg/g creatinine). Female preferential effects have been evident from the 1999-2004 NHANES data where the dose-response relationship of Cd exposures and prevalence rates of peripheral artery disease showed U-shaped only in women who never smoked.[34] The U.S. NHANES III data have indicated that prevalence rates of pre-diabetes and diabetes increased proportionally with increasing urinary Cd levels.[35] The risk estimates for abnormal fasting glucose and diabetes were 1.48 and 1.24 for pre-diabetes and diabetes, respectively, when comparisons were made for urinary Cd levels of <1 with those between 1.00-1.99 μg/g creatinine. These increased to 2.05 and 1.45 when comparisons were for urinary Cd <1 with those of ≥2 μg/g creatinine.[36]

Mechanisms underlying cadmium toxicity
Oxidative stress and inflammation

Urinary Cd levels as well as blood lead levels of participants in the U.S. NHANES III were associated with oxidative stress, reflected by serum levels of γ-glutamyltransferase. [37] Also in the U.S. NHANES III study, urinary Cd levels were associated with the prevalence of blood C-reactive protein ≥2.2 mg/L and fibrinogen ≥10.35 μmol/L, markers of inflammation and known risk factors for cardiovascular disease.[38] In another study, blood Cd levels of men ≥40 yrs in 1999–2004 NHANES increased with increasing levels of serum alkaline phosphatase (a marker of liver inflammation), increased prevalence of diabetes and hypercholesterolemia. [38] Mechanistic studies in human proximal tubular epithelial cells have provided evidence linking Cd-induced oxidative stress with mitochondrial dysfunction, resulting in a fall in ATP output together with over-production of reactive oxygen species.[39] Of note, mitochondrial dysfunction has been implicated also in the development of insulin resistance, an important feature of type-2 diabetes.[40] It has been suggested that targeting mitochondria to protect mitochondrial function could prevent and ameliorate various diseases associated with mitochondrial dysfunction.

Epigenetic alteration (DNA methylation state)

Data from *in vitro* studies using the rat liver epithelial cell line (TRL1215) has implicated Cd-induced epigenetic effects in cancer development.[41] Cd at 2.5 μM concentration has been shown to cause the TRL1215 cells to undergo malignant transformation after 10 weeks of exposure.[41] The Cd-exposed TRL1215 cells exhibited phenotypic characteristics of transformed cells; hyperproliferation, increased invasiveness, and decreased serum dependence. In addition, increases in activity of DNA methyltransferase and DNA methylation were observed, providing for the first time suggestive evidence for epigenetic effects of Cd.[41] In

a subsequent study, carcinogenic potential of Cd at lower level (1.0 μM Cd, as $CdCl_2$) was demonstrated using the same cell line (TRL1215), but with exposure duration extended from 10 weeks to 28 weeks.[42] The resultant Cd-transformed TRL1215 cells were hyperproliferative with a growth rate about 3-fold greater than passage-matched control cells. Upon inoculation into nude mice, those transformed cells produced highly aggressive tumors. Interestingly, Cd at its carcinogenic dose level 1μM did not increase the levels of reactive oxygen species within 24-hr exposure. But it instead caused 10-fold increases in expression levels of the oncogenes c-myc and c-jun, consonant with increases in DNA-binding activity of the transcription factors AP-1 and NF-ϰB. Over-expression of oncogenes and loss of growth control appeared to be critical in carcinogenicity of prolonged, low-level Cd exposure as oppose to increased oxidative stress, frequently observed with higher exposure levels.[42,43] Taken together, these data indicated that prolonged exposure to low-level Cd caused DNA hypomethylation and proto-oncogene activation.

Prolonged exposure to low-level Cd has been shown to induce cancer transformation also in human cell lines, namely the human uroepithelial cell line UROtsa, prostate epithelial cell line, normal breast epithelial cell line, MCF-10A.[44-46] Carcinogenic potential of Cd demonstrable in normal breast epithelial cell line, MCF-10A has prompted further research into a role played by environmental exposure to Cd in the high incidence rates of breast cancer in Long Island, New York.[47] In support of the conclusion derived from rat liver epithelial cells, epigenetic modification of genes, critical in carcinogenesis (oncogenes, tumor suppressor protein) appeared prominent in Cd-induced transformation of the human prostate epithelial cell line and normal breast epithelial cell line, MCF-10A.[45,46] Cd-induced proliferation of the human K562 erythroleukemia cells has been shown to be associated with global DNA hypomethylation, possibly resulted from the ability of Cd to cause depletion of cellular glutathione.[48,49] Influence of Cd exposure on other human genes identified to be critical in cancer development via a range of epigenetic mechanisms, e.g., histone modifications, microRNA expression remain to be explored.[50,51]

Use of chelating agents in the management of cadmium toxicity

In occupational exposure scenarios, intervention for toxicity from any metal includes removal from exposure sources.[52] For the general population, however, primary public measures are to be implemented to minimize environmental pollution and the food-chain transfer of Cd. Effective therapeutic chelation for reducing Cd body burden may also be useful, given the emerging evidence that toxic effects may occur in adults with low-level exposures to Cd, resulting in urinary Cd as low as 1μg/g creatinine. Indeed, the idea of use metal chelation to reduce blood lead levels followed the awareness that toxic effects (impaired cognitive and behavioural development) may occur in infants and young children with low-level exposures to lead, resulting in blood lead levels as low as 10-15 μg/dL. Synthetic chemicals including dimercaptosuccinic acid (DMSA), known also as succimer have been approved by the Food and Drug Administration for use in chelating of lead. Promising chelating agents for Cd are substituted dithiocarbamate compounds with a pair of sulphur atoms. For example sodium N-(4-methoxybenzyl)-D-glucamine dithiocarbamate has been shown to be effective in removing Cd from tissues of exposed animals, but its efficacy in humans has not been demonstrated.[52]

Alpha-lipoic acid and cadmium chelation

Alpha-lipoic acid (1, 2-dithiolane-3-pentanoic acid or thioctic acid) is one of the common ingredients of dietary, multivitamin and anti-aging supplements and mitochondrial nutrient cocktails.[40,53,54] Plant sources of LA include spinach and broccoli.[55] LA and its reduced form (dihydrolipoic acid, DHLA) exhibit antioxidant properties. DHLA has been shown to scavenge superoxide and peroxyl radicals while regenerating glutathione, thioredoxin, vitamin C and recycling of vitamin E.[56] LA is ranked the highest in antioxidant potency, compared with other sulfur-containing antioxidants, namely cysteine, methionine, taurine, glutathione, N-acetylcysteine, mercaptopropionylglycine, and organosulfur compounds of garlic, namely diallylsulfide, diallyldisulfide and diallyltrisulfide.[57] Recently, LA has been shown to improve body status of glucose and vitamin C although it has long been used in the treatment of diabetic neuropathy.[53,58] Further, therapeutic efficacy of LA plus N-acetylcysteine has been shown in clinical trials; the SYDNEY trial, SYDNEY 2 trial, and ALADIN III study, in diabetes with albuminuria, and in women with polycystic ovarian syndrome.[59]

In addition to their remarkable anti-oxidant properties, LA and DHLA have been shown to bind a number of metal ions under *in vivo* and *in vitro* conditions. LA can bind to Cu^{2+}, Zn^{2+} and Pb^{2+}, but not Fe^{3+}, whereas DHLA can bind Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3+}.[60] Based on these metal binding properties, it has been postulated that chelation of redox active metals such as Fe^{3+} and Cu^{2+} by LA and DHLA can reduce the levels of free radicles generated from the Fenton's reactions, catalysed by those redox metals. A positive effect DHLA in Alzheimer's disease is thus thought to be due DHLA-mediated chelation of Fe^{3+} and Cu^{2+} in the brain, leading to lowering free radical damage.[61] Further clinical application of α-lipoic acid in vascular disease, hypertension, and inflammation has been suggested, based on its ability to enhance the activity of endothelial nitric oxide synthase and phase II enzymes while causing NF-kappa B repression, leading a reduction of MMP-9 and VCAM-1 expression.[53] Whether LA/DHLA effectively binds to and removes Cd^{2+} ions remain to be elucidated. The ability of α-lipoic acid to regenerate glutathione suggests its potential role also in the maintenance of DNA methylation state, shown to be affected by Cd exposure and some nutritional deficiencies of the amino acid methionine, folate and vitamin B12.[48,49,62] Whether α-lipoic acid prevents Cd effects on DNA meth-

ylation merits an investigation, which should be included as one of the criteria in the evaluation of health benefit and Cd chelation efficacy of α-lipoic acid.

Concluding remarks

Exposure to Cd, especially of dietary origin, is perhaps one of the least expected and so the least recognized factor in the rising incidence of cancer and chronic diseases (diabetes, chronic kidney disease) worldwide since the guidelines for safe levels of intake have been established.[27-29] Those guidelines, however, are challenged by data from numerous epidemiologic studies, observing a range of adverse health effects in the general population without obvious exposure (non-smoking and non-occupationally exposed).[63-65] Avoidance high-Cd food is important, but so is an adequate intake of dietary essential metals (iron, calcium, and zinc) to maintain body status of essential metals. However, dietary exposure minimization is pivotal, due to a lack of active biochemical mechanism for Cd excretion. Plant-based functional foods along with a well-balanced diet rich in fruits and vegetables can have a place in the management of Cd toxicity in the general population. However, it is noteworthy that the therapeutic efficacy of vegetables such as spinach, broccoli, shown to have the ability of chelate Cd and to oppose its epigenetic effects, could not be demonstrable in the presence of Cd contamination of food supply. This may be one of the explanations for a lack of efficacy of fruit and vegetable consumption in reducing overall cancer risk in European Prospective Investigation into Cancer and Nutrition (EPIC) and the randomized trials and population studies using natural antioxidants in management of blood pressure and cardiovascular risk.[66] Long-term management of Cd in the environment and in agriculture is also required to minimize the food-chain transfer of Cd, notably from use of phosphate fertilizers. Avoidance of further soil Cd contamination needs much more awareness of Cd levels in phosphate fertilizers, and in mining waste and waste-water. The persistence of this metal in the environment requires a long-term management approach to minimization of human exposure through environmental management and maintenance of lower Cd levels wherever possible.

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Role of phytochemical antioxidants in experimental models of oxidative stress-induced vascular dysfunction

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In the state of oxidative stress, the production of reactive oxygen species (ROS) exceeds the available antioxidant defense systems. Excessive production of ROS is an essential mechanism underlying pathogenesis of vascular dysfunction and the progression of cardiovascular diseases (CVD). Endothelium-derived nitric oxide (NO) is a paracrine factor that controls vascular tone, inhibits platelet function, prevents adhesion of leukocytes, and reduces proliferation of the intima. An enhanced inactivation and/or reduced synthesis of NO is seen in conjunction with risk factors for CVD, including hypertension, diabetes mellitus, atherosclerosis, stroke, ischemic heart disease, congestive heart failure and renal disease. There are several enzyme systems that can potentially produce ROS in the vessel wall, with four systems being of major importance. These include NADPH oxidase, xanthine oxidase, dysfunctional endothelial NO synthase (eNOS), and enzymes of the mitochondrial respiratory chain. A dominant mechanism reducing bioavailability of vascular NO may be related to its rapid oxidative inactivation by the superoxide ($O_2^{\cdot-}$). Moreover, increased ROS concentrations reduce the amount of bioactive NO by chemical inactivation to form toxic peroxynitrite. These highly reactive molecules oxidize lipids resulting in cellular injury and vascular oxidative stress. A substantial body of evidence suggests that dietary polyphenolic antioxidants have a protective role against CVD. Since vascular dysfunction is an early feature of CVD, improvement or augmentation of vascular function may prevent the development of CVD. In the experimental models of oxidative stress-induced vascular dysfunction, including hemolytic anemia, endotoxemia, hypertension or diabetes, phytochemicals such as quercetin, curcumin and tetrahydrocurcumin could reverse the abnormal vascular function. These protective effects are associated with the suppression of ROS generation and restoration of cellular redox balance. The effect of phytochemical antioxidants may also involve with several antioxidant-responsive genes, which suggest the cytoprotective effect apart from direct antioxidant activity. Overall, our observations suggest that plant foods rich in phytochemicals may exert the cardioprotective effects and provide a basis for further investigating their therapeutic potentials to improve vascular function and reduce risk of cardiovascular events.

***Keywords**: antioxidant, oxidative stress, phytochemicals, vascular dysfunction*

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Symposium 4: Assisted conception: an integrative approach of reproductive sciences and medicine

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Assisted conception: milestones and clinical applications

Supat Sinawat

Introduction

Significant progress has been documented in the field of Reproductive Sciences and Medicine during the past two decades. This was owing in part to the successful application of gamete and embryo culture to medical, veterinary, and biotechnology practices, and, of no doubt, to the needs of today's society. Assisted conception is a good and very precise example of how scientists working in the field of Reproductive Sciences can offer their expertise and integrate the knowledge obtained from laboratory research to fulfill the needs of people seeking medical assistances from Reproductive medicine specialists due to infertility problems. Advances in this special integrated field not only help many infertile patients to have a child of their own, but also offer many more potential applications such as pre-implantation genetic diagnosis, gene therapy, and stem cell research which will eventually lead to a better quality of life.

The milestones of assisted conception

Assisted conception or Assisted Reproductive Technology (ART) encompasses all techniques that involve direct manipulation of sperms and/or oocytes outside of the body. In medical science, assisted conceptions have been developed primarily to alleviate sterility, while in agricultural sciences the growing needs of the booming world population has provided the impetus to improve the efficiency of livestock production. The earliest documented use of assisted conception was in 1783 when Spallazani delivered pups from an artificially inseminated bitch, but it was not until the 1900s that the Russian School of Ivanov developed artificial insemination techniques to be used in horses, cattle, and sheep. The value of artificial insemination in farm animals depends upon the fact that the male ejaculate contains millions of spermatozoa sufficient to inseminate dozens of females.[1]

A major progress in this field was made in the late 1940s, when the team led by Chis Polge in Cambridge, UK, developed techniques to freeze and store animal spermatozoa. Moreover, development of methods to isolate and manipulate the female gametes had been established during the same period of time. In vitro maturation of mammalian oocytes was reported by Pincus over 60 years ago when it was observed that the primary oocyte of the rabbit resume meiosis spontaneously when liberated from its follicles and placed in a suitable culture medium. In 1968, Joe Sreenam in Ireland observed nuclear maturation in vitro in bovine oocytes recovered from slaughterhouse cattle. Although experiments on animals have traditionally preceded human studies, this has not always been the case in reproductive sciences, where many of the new techniques and breakthroughs have been made using human gametes.[1]

Experiments in which sperms were injected into eggs took place around 1960s. These were primarily designed to investigate the early events of fertilization. Ryuzo Yanagimachi demonstrated that isolated hamster nuclei could develop into pronuclei after microinjection into homologous eggs, and a similar result was obtained when human spermatozoa were injected into a hamster egg. These experiments indicated that during activation of mammalian oocytes, membrane fusion events may be bypassed without compromising the initiation of development. The experiments not only provided information on the mechanism of fertilization, but led to a new technique in clinical embryology.

The first and still most common form of human assisted conception is in vitro fertilization (IVF). There are, however, a number of other related techniques within the realm of ART. The success of modern ART has completely revolutionized both the evaluation and treatment of infertility. Some traditional treatments have been rendered obsolete and others now have only limited applications because ART is simply more effective. The trend is certain to continue.[2]

Indications for in vitro fertilization

In vitro fertilization (IVF) was first developed as a mean to overcome infertility resulting from irreparable tubal diseases, but it is now applied much more broadly for the treatment of almost all causes of infertility. IVF is most certainly when the method offers the means to overcome one or more specific obstacles not otherwise amenable to treatment; severe tubal disease resulting from previous infection or advanced stages of endometriosis and severe male factor infertility are the most obvious examples. IVF is also often the best treament6 for couples with multifactor infertility because the method can effectively address all contributing causes at the same time. IVF is a legitimate treatment option for women with age-related or otherwise unexplained infertility and represents the treatment of the last resort for those in whom all other treatments have failed.

Nowadays IVF has been applied to solve much broader fertility problems. In women with premature ovarian failure and healthy women beyond the normal reproductive age, IVF using oocytes from a young donor is highly successful. For women with normal ovaries but no functional uterus (mullerian agenesis, previous hysterectomy) and those with medical disorders in whom pregnancy would pose a serious

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health risk, IVF with embryo transfer to a gestational surrogate still offers the possibility of genetic offspring. In couples who carry autosomal recessive or sex-linked genetic disorder or a balanced chromosome translocation, IVF and PGD can be used to avoid the risk of deliveri9ng an affected child. There is growing interest in the use of IVF with PGD to identify and exclude aneuploid embryos in women of advanced reproductive age, those with history of recurrent pregnancy loss, and the women with repeated unexplained IVF failure despite transfer of otherwise normal-appearing embryos.

In vitro fertilization: a clinical perspective

In nature, efficient reproduction relies on the synchronized behavior of animals, the synchronized physiology of their reproductive organs and the synchronized interaction of the male and female gametes. This fundamental principle of synchronization has to be respected in assisted conception, irrespective of the technique involved.

In vitro fertilization (IVF) involves a sequence of highly coordinated steps beginning with controlled ovarian hyperstimulation with exogenous gonadotropins, followed by retrieval of oocytes from the ovaries under transvaginal ultrasound guidance, fertilization in the laboratory, and transcervical transfer of embryos into the uterus.

The first child resulting from IVF was born in 1978.[3] Over the years since, the techniques involved have been both highly refined and greatly expanded. Assisted conception now includes methods for assisted fertilization by intracytoplasmic sperm injection (ICSI) using sperm isolated from the ejaculate or obtained by microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE), assisted embryo hatching, and preimplantation genetic diagnosis. In most cases, IVF is used to help an infertile couples conceive their own biological child, but donor sperm, donor oocytes, and gestational surrogates can also be used, alone or in combination, as circumstances require.

Other form of assisted conceptions involve the tubal transfer of oocytes and sperm (gamete intrafallopian transfer, GIFT), zygotes (zygote intrafallopian transfer, ZIFT) or embryos (tubal embryo transfer, TET) via laparoscopy. In past years, these more invasive techniques offered certain advantages over traditional IVF for some infertile couples, but no longer. Consequently, they now have only limited indications.

Results of IVF

IVF outcomes have improved steadily throughout the years since its introduction into clinical practice. Over the 5-year interval between 1996 and 2001 in the US, live births per transfer for ART cycles using fresh nondonor oocytes or embryos have risen gradually for all age groups. Live birth rate per transfer have risen from 33.6% to n41.1% for women under age 35, from 29.0% to 35.1% for those ages 35-37, from 21.6% to 25.4% for women 38-40, from 11.5% to 14.5% for those ages 41-42, and from 5.4% to 6.7% for women over age 42.[4]

Success rates of IVF vary somewhat with diagnosis or cause of infertility. Couples with tubal factor infertility, ovulatory dysfunction, endometriosis, male factor infertility, or unexplained infertility had above average success rates. The lowest success rates were observed for those with a diminished ovarian reserve. Couples with uterine factor infertility, or multiple infertility factors had below-average success rates. In all age groups, success rates are higher for women with a previous live birth and those in their first ART cycle than for nulliparous women and those with a previous failed ART cycles.[5]

Risks of IVF

IVF increases the risk of multiple pregnancy, ectopic pregnancy and the ovarian hyperstimulation syndrome. The risk of multiple pregnancy is increased substantially in ART cycles. In 2000, 355 of all births in the US resulting from ART were multiples, a rate 10 times higher than the 3% multiple infant-birth rate in general population. The risk of ectopic pregnancy is increased at least 2 –fold in women who conceive via ART. [6] The risk of ovarian hyperstimulation syndrome increases with exposure to hCG, higher dose of exogenous gonadotropins, and high serum estradiol level. Earlier worries about a possible link between ovarian cancer and ovulating-inducing agents have largely declined but still linger.

Offspring from IVF

Studies of the offspring resulting from IVF have raised concerns that the children may be at increased risk for birth defects, prematurity, low birth weight, delayed neurological development, and both genetic and epigenetic abnormalities.[7]

Most studies of children born after ART have found a prevalence of congenital malformation similar to that in the general population (2-3%) [8-10], an Australian study, however, observed a 2-fold increased risk of major birth defects among children conceived via conventional IVF or ICSI, compared to that in a matched population of children who were naturally conceived. [11] Specific anomalies that appear over-represented in ART children include neural tube defects, alimentary atresia, omphalocele, and hypospadias.

Several studies have indicated that infants conceived via ART are at increased risk for prematurity and low birth weight. [12] Some investigators have suggested that altered epigenetic programming of gene expression in pregnancies resulting from ART may adversely affect the fetal growth. [13]

An increased prevalence of neurological sequelae has been observed in children born after IVF, especially cerebral palsy, largely attributed to higher frequency of multiple pregnancies, prematurity, and low birth weight.

The incidence of sex chromosome abnormalities appears to be modestly increased (approximately 1%) in children born after ICSI, probably reflecting the increased

prevalence of similar abnormalities in men with severe oligospermia or nonobstructive azoospermia rather than the ICSI procedure itself. There is some evidence to suggest that children resulting from ART also may be at increased risk for disorders resulting from imprinting errors during early embryogenesis in which a specific parental allele is preferentially expressed. Preliminary data suggest that the prevalence of Angelman syndrome (mental retardation, delayed motor development, poor balance, abnormal movement, absent speech) and Beckwith-Wiedemenn syndrome (macrosomia, macroglossia, midline abdominal wall defects, predisposition to embryonal cancer), both resulting from imprinting defects, may be increased in children born after ART. [14]

Conclusion

Assisted conception or Assisted Reproductive Technology (ART)) involves a sequence of highly coordinated steps. The significant progress in this field during the past decades results from all the efforts and time both scientists and clinicians working in this specific field have been attempted to gather more information necessary to improve the success of this method. ART, therefore, represents a very good example of a teamwork and integration of reproductive sciences research and their clinical applications. Further development in this field will certainly bring up more breakthrough and potential use of these techniques.[15]

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Assisted conception: present status and future applications

Teraporn Vutyavanich

Assisted reproductive technology (ART) is defined as any fertility treatments in which oocytes and/or embryos are handled outside the female body. In general, it involves surgically aspirating oocytes in natural or stimulated cycles, inseminating them in the laboratory, and transferring the embryos to the uterus. ART covers a broad range of procedures, such as *in vitro* fertilization (IVF), gamete intrafallopian transfer (GIFT), intracytoplasmic sperm injection (ICSI), and cryopreservation of oocytes and embryos. ART is now widely practiced around the world and more than 4 million babies have been born to date. ART is used to treat both male and female infertility, when conventional treatments fail or when there is no other treatment option. At present, it is also employed in fertile couples, who are carriers of certain genetic diseases, such as beta-thalassemia. Embryos obtained from IVF can be biopsied in order to obtain specific genetic and chromosome information by different molecular techniques, such as polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH). This technique, known as preimplantation genetic diagnosis (PGD), also enables the selection a child (designer baby), who is a genetic match to an affected sibling, for the purpose of stem cell donation. ART is a good example of an integrative approach of reproductive physiology and medicine. ART will not be possible without an existing knowledge of ovarian physiology, physiology of sperm capacitation and fertilization process. On the other hand, developments in clinical ART have provided major advances in our understanding of the physiological process of fertilization and embryo development. ART has opened our door to new frontiers in reproductive science and medicine, such as the field of embryonic stem cell and oncofertility. It is not surprising that Professor Robert Edwards, the British physiologist, was awarded the 2010 Nobel Prize in Physiology/Medicine for the development of human in vitro fertilization therapy. Despite many recent advances in this specialty, the outcome of ART continues to be relatively inefficient. In most circumstances, there is no explanation other than the failure of embryos to implant. In some cases, implantation failure occurs repeatedly and poses a dilemma and a challenge to the practicing clinicians. Promising future treatment approaches will include ways to enhance endometrial receptivity, improve oocyte and embryo quality and select the best single embryo for transfer. The creation of human gametes (oocytes and embryos), through stem cell technology, will overcome age, which is currently the limiting factor of ART.

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Symposium 5: New approaches and tools in neuropsychological disorders

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Cancer stem cells in children brain tumors from bench research to bedside application

Wiyada Punjaruk

There are many neurophysiological disorders including functional or non-functional disorders detected by recent advanced equipments. However, one of problematic disorders of central nervous system occurred in both adult and children is brain tumors. Particularly in children, it is the second most common cancers occurred after leukemia. Currently, the response of treatment is poor and the recurrent rate after complete course of therapy is high. These result in poor prognosis and low survival rate. Some children brain tumors however seem to have high survival rate but they still have permanent sequelae resulting from surgery or chemotherapy or radiation especially involving in cognitive functions. Recent studies have revealed that cancer stem cells (CSCs) exist in many malignant diseases including brain tumors. Additionally, it is proposed that these cells may survive following chemotherapy and radiation, and hence contribute to tumour relapse and chemotherapeutic resistance. Therefore, it is essential to understand the roles and drug resistant mechanisms of CSCs. The required processes of studying CSCs begin with researching on primary cultured cell lines derived from patients diagnosed with brain tumours then animal models based on the evidences from cell line study. Finally, therapeutic trials in patients will be performed after successful studies in both cell lines and animal models. Therefore, knowledge of cancer stem cells acquired from bench research through bedside application will be an important key to open a new door to hopefully cure many children brain tumors in the future.

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Symposium 6: Exercise, lifestyle modification, nutrition and meditation

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Dimensions of CAM in exercise, lifestyle modification, nutrition and meditation

Alex Hankey

Complementary and alternative (CAM) systems of medicine are now recognized to add valuable dimensions to conventional medical treatment. Asia boasts many such systems - those of East and South East Asia, as well as South Asia's, brought into India's health policy by its Ministry of Health's Department of AYUSH, governing Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy. Ayurveda, Yoga and Siddha date from antiquity, originating in Vedic culture, dates for which are now accepted to precede the end of the last ice-age (5,000 years ago).

Asia's traditional systems offer dimensions connected to healing. Prana/Shakti (India), or Qi (TCM), play central roles in health promotion and restoration. In contrast to western, 'allopathic' medicine's obsession with disease, Asian systems recognize health maintenance and promotion as health care's primary activities. Central to prevention is the concept of 'life in accordance with natural law' - for each person not to engage in activities causing their physiology unnecessary strain and damage.

Exercise, lifestyle modification, nutrition and meditation represent important aspects of health promotion in traditional CAM systems: an early morning walk to 'shake off dull sloth' or more vigorous exercise to improve circulation; maintaining daily routine and activity at levels appropriate to one's own physiology; ingesting the right food at the right time and place, appropriate to a patient's needs; and maintaining all mental function in accordance with health promotion, are all important in traditional health care. These will all be expounded in detail, with particular reference to criteria in South Asia's traditional systems.

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Possible role of mild enjoyable exercise for improving cognitive functions in the elderly

Hideaki Soya, Kazuki Hyodo

We are still arguing about a cure for Alzheimer's disease (AD). Recent reports suggest that enriched environment and physical activities might provide an effective strategy to delay AD's onset, most likely by promoting neuronal plasticity and survival. Although exercise intervention reduces the risk for dementia among elderly subjects, it is not clear whether their cognitive functions could be improved and/or progression to AD could be delayed. We thus investigated whether 1-year dance calisthenics of Furi-furi guppa, a mild hedonic exercise intervention, could enhance cognitive and physical functions in an elderly population of Tone town in Ibaraki, Japan. Two hundred and nineteen subjects with normal cognitive function and MCI, extracted from 2,730 eligible subjects, at ages 65 years and above, participated in the exercise intervention trial. The 1 year exercise intervention trial comprised of a home and club-based program (6 times/month) of Furi-furi guppa. Changes in memory functions, muscle strength, aerobic capacity, reaction time, daily energy expenditure and other biochemical parameters such as urinary free cortisol were measured. After the exercise intervention, a high adherence rate of 70% was noted. Significant improvements in memory and physical functions, and decreased urinary cortisol levels were observed. Memory function was positively correlated with mean energy expenditure and negatively correlated with urinary cortisol levels. A mild dance calisthenics intervention at the community level can be effective in abating stress and cognitive decline among elderly persons, especially within the MCI cases. This is strongly supported by the basic animal research that even mild exercise activates the hippocampus leading to increased BDNF (brain-derived neurotrophic factor) and neurogenesis. Thus, it is tempting to suggest that several forms of mild exercise, dance, calisthenics including oriental physical culture should be organized to develop a new exercise prescription with an aim to enhancing the body and mind, especially our cognitive functions.

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Yoga and holistic health

P. Nazini

Introduction about yoga

Yoga is an ancient art based on a harmonizing system of developed for the body, mind and spirit. It is a practical aid, not a religion. The continued practice of yoga will lead one to a sense of peace and well being and also a feeling of being in harmony with one's environment.

The word 'yoga' comes from the Sanskrit root "yuj", which means. "To Join" or "To Yoke". Yoga is one of the six systems of Indian Philosophy. The classical form of yoga, based on the text ascribed to patanjali, became known in the middle ages as Raja yoga or "Royal Yoga". Other forms of yoga also developed, which might be followed together with, or independently of, the classical yoga. Among these the practices of Hatha Yoga have become famous throughout the world, and the term Yoga is often used to denote them. Hatha yoga seems to be a late development in Hinduism, and the earliest texts on the subject date from little before the Muslim invasion. It is closely connected with Tantrism, though many practitioners of Hatha Yoga are not Tantrists.

History of yoga

The history of Yoga can be divided into the following four broad categories.

- Vedic yoga
- Pre classical yoga
- Classical yoga
- Post classical yoga

These categories are like static snapshots of something that is actuality in continuous motion- the "March of history".

Vedic yoga

The Sanskrit word Veda means "knowledge", while the Sanskrit term Rig, means "Praise". Thus the sacred Rig Veda is the collection of hymns that are in praise of higher power.

Pre classical yoga

This category covers an extensive period of approximately 2,000 years until the secondary century. Pre classical yoga comes in various form and guises. The earliest manifestations were still closely associated with the Vedic sacrificial cultural, as developed in the Brahmans and Aranyakas.

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Classical yoga

This label applies to the eightfold yoga also known as Astanga –yoga or Raja Yoga taught by Patanjali in his Yoga-Sutra. This Sanskrit text consisted of 196 Sutras or aphorisms, which have been commented on over and over again through the centuries.

The great sanint Patanjali believed that each individual is a composite of matter (Prakriti) and spirit (Purusha). He understood the process of yoga to bring about their separation, thereby restoring the spirit in its absolute purity.

Post classical yoga

This is again a very comprehensive, which refers to all those many types and schools of Yoga that have sprung up in the period after Patanjali's Yoga Sutra and that independent of this decisive work. In contrast to Classical Yoga, Post classical Yoga affirms the ultimate unity of everything. This is the core teaching of Vedanta, the philosophical system based on the teaching of the Upanishads.

Definition of yoga

Modern terms can rightly be designated as the technique of holistic living – man understanding himself to be, not a part, but the whole of Truth, Existence, Knowledge and Bliss.

Practice can increase our lung capacity and respiration, improve our ability to resist stress, reduce body weight and girth, decrease cholesterol and blood sugar levels and thus stabilize, restore and vitalize the body's natural systems.

The concept of yoga

Yoga is an ancient discipline. It is recognized as one of the most important and valuable gifts of our culture. The modern era, with the developed of science and technology provides man more comfort for his basic necessities. But with these comforts man faces lot of problems, which cannot be solved only by the above facilities. Today the world is looking for solutions to solve the menacing problems of unhappiness, restlessness, emotional imbalance, hyper activity, tension, stress, etc.

Yoga improves posture, increases the intake of oxygen, and enhances the functioning of the respiratory, digestive, endocrine, and reproductive and excretory systems. Its effect on the emotions is equally beneficial by calming the mind, attuning us to the environment and diminishing insomnia caused by mental restlessness. Yoga is highly recommended for people in competitive, stressful working environments, for those who suffer from headaches, back and shoulder aches, allergies and asthma. Yoga also cures behavioral disorder, nervous breakdown and manic depression. The regular practice of yoga helps us to accept

whatever physical or mental conditions we might be suffering from by increasing our immediate sense of well – being, concentration, and calmness. Much healing can be done, but it takes practice and consistency.

We all have the capacity to self- destruct, particularly if things go wrong. The yogic mentality is that life is a tremendous gift and we have to take responsibility for it. Yoga gives you the capacity to face up to life's challenges. Similarly, when you respect your body, you tend to do things that will enhance its vitality. Part of yoga practice is deep breathing, which helps to make the body more alkaline. The acid-alkaline ratio is crucial to good health. It should be 80 % alkaline, 20 % acid. Over acidity can be harmful for bones and tissues, leading to fatigue, dulled mentality, headaches, depression and arthritis. Refined carbohydrates, animal proteins, coffee and alcohol, as well as stress and pollution are all acid forming.

Yoga works on a psychological level too. In a yoga position, one should concentrate on a total awareness of our energy and how it flows. One should learn how body and mind works together. Almost all exercise can be beneficial depending on the intent and body condition; practicing yoga ultimately leads towards long-term health and well being.

Yoga is a science and art

Basically speaking, creativity is a fact of an imaginative mind. Creativity is dormant in every human being and it is possible for all aspirants to unfold, develop and channelize this creative faculty effectively.

The role of creative faculty in art and science has now been understood by the modern man probably more than ever before. The emergence of creativity as the core of technology has added aesthetics or a new dimension in the field of science. Basically, art has been impregnated into the science.

Similarly, in the field of starts, music, sculpture, painting, etc, a scientific outlook is coming up. Trend in modern terms tries to understand the science of arts and the arts in science. Hitherto, science and arts were considered as their mutual interaction, in fact, this has been so in the history of the growth of science, whenever a new dimension of mind, far deeper and subtler. Man is curious to get to the subtle substratum, from where creativity emerges. This has now become the direction of search.

Yoga, a conscious and systematic process to accelerate the growth of human mind, is now emerging as a new tool in this search. Yoga, in general, and meditation, in particular, is providing man a means to reach the subtle layers of mind. It has been shown through the experimental results on meditation; that knowledge and creativity are structured in subtler layers of mind or deeper states of consciousness (transcendental state.). These creative and critical faculties of mind lie hidden in these higher states of consciousness. Thus, the foundation of arts and science are now being found in deeper states of our consciousness. Hence, yoga has brought a breakthrough in unraveling the hidden dimensions of mind. All those seeking to develop grater and creative faculties now have Yoga as a new tool and millions of people all over the world have taken to the study and practice of yoga and are reaping its benefits.

The eight steps of the yogic path

These eight steps of the yogic path are meant to be accomplished sequentially. That is, one masters the first, and adds the second. When the second is mastered, the third is added, and so on.

The first Five or 'external' stages are

Yama or 'restraint'

The path begins with self-discipline adoption of a basic moral code of non-karmic or 'unselfish' activity. The yogi forsakes stealing, lying, cheating, killing, and other exploitative and self-gratifying behaviours.

Niyama or'purity'

Purity involves both hygiene and diet. In terms of hygiene, radical ablutions or cleansing rituals are performed, such as swallowing a length of gauze and pulling it back out again, in order to scour the intestinal tract. Thus hygiene goes beyond the superficial conception of cleanliness which governs ordinary life. Diet is also important, since outermost sheaths are composed of the food that we eat. Dense foods such as meant are to be avoided, and subtle, refined foods are to be preferred. Also important purity, but it is seen as a hygienic concern, and not a dietary one.

Asana or 'postures'

The twisting, bending, and stretching that are commonly associated with the practice of yoga serve a number of purposes. The holding of postures prepares the body to sit for long periods of time in meditations, enables the overcoming of the boredom reflex, and is held to stimulate the endocrine system and thus to be important, since the endocrine system affects our emotions: this stage of yoga begins to affects emotional as well as the physical sheaths.

Pranayama or 'breathing exercise'

Prana is the life force which enters the body with the breath and which is metabolized from the foods we eat. Breathing exercise improve the ability of the body to metabolize prana. Also, since breathing affects emotions, breathing affects emotions, breath work helps to regulate and refine the emotional sheath. Finally, breathing also represents a bridge between those physiological functions which we believe we can control (*involuntary*). Adept yogic claims to be control metabolism, reflex, and brainwave activity events slow or virtually stop the heartbeat.

Pratyahara or 'sensory withdrawal'

At this stage, yogi is able to use the power of concentration to withdraw attention and identification from the outermost, physical, 'external' sheaths. This means that sensory input is blocked out or ignored through an effort

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of will. The only sound one hears is the pounding of the heart, and this explains why a yogi might want to slow or stop the heartbeat, in order to establish true peace and quiet and facilitate inwardness.

The last three or 'internal' stages are;

Dharana or 'concentration'

Concentration in this sense involves what is described as *single-pointedness*, that is, the fixation of mind, body, and spirit on a common focal point. Here, the image of the *third eye* is invoked to suggest the strengthening of spiritual vision to the point where it is capable of sustaining a single object for long period of time, like an eye staring at an object.

Dhyana or 'meditation'

Dhyana refers to meditation, or a sense of radical self-awareness. To return to the metaphor of the third eye, once it has been trained to stare unblinkingly at a single object for a long period of time, it then turns inward upon itself, watching itself watch itself. This awareness takes place without judgment or evaluation, and drives a wedge between our experience and our Self. We watch or 'witness' our own experience as though it were only virtually real, as though it were a drama or play. We cease to identify with it.

Samadhi or 'bliss-trance

This condition is one of complete effacement of individuality. One no longer identifies with one's body or ego: one's actions are selflessly motivated and non-karmic. This virtually guarantees that liberation will occur with death, which will take place once the consequences of past karmic action have been borne.

Yoga and diet

It has been proved that diet plays a vital role in determining the qualities of man, and a yogi has to carefully analyze the diet which he consumes in his day to day life. He can select his food by understanding the basic concept about the effect of the various kinds of food on our system. A 'Balanced Diet ', therefore, according to yoga, is that diet which restores balance at all levels. Such diets could aid in a Holistic Way of Living. Yoga classifies food into 3 categories, similar to the classification of human being into predominantly a) Tamasic b) Rajasic and c) Sattvic.

Tamasic food – it is sale, more or less spoiled food, containing foul odor, artificial additives, and which is not all useful to nourish either body or mind. They make the body dull, lazy and drowsy and reduce our immune power, filling the mind with dark emotion such as anger and greed. Tamasic food items include alcohol, tobacco, onions, garlic and fermented food as vinegar.

Rajasic food- It is very hot natured, spicy, bitter, sour, pungent, dry and excessively salty. Such food items are real enemy of mind body equilibrium. They function as body stimulants and exit the passion, making the mind restless and uncontrollable. Food which is cooked a great deal to increase its taste appeal, that which stimulates and activates the nervous system, speeds up metabolism, e.g. coffee, tea, tobacco, green chilies and pepper are considered Rajasic but dried red chilies tend to be more Tamasic. High quality wines are Rajasic.

Sativc food- It is the purest diet and is more suitable for yoga practitioner. It nourishes the body and maintains it in a peaceful state. It also purifies the mind, enabling it to function at its maximum potential. The satvic food consists of fresh fragrant and tasty items. It includes cereals, fresh fruits and vegetables, milk and milk products, nuts and honey. Those foods which increase the life (Purity), strength (health) and happiness (cheerfulness and good appetite) are termed Satvic.

All the different types of food eaten by man in the world have been classified and brought under four types on the basis of their physical properties. They are Savory, Greasy, Firm and Cordial types of food. Diet can influence the mind and change the personality, but a strong mind can digest the most Tasmic food and still live very healthy.

Integrated approach of yoga therapy

In yoga therapy it is vital that we take into consideration all of the following aspects that are part of an integrated approach to the problem. These include a healthy life nourishing diet, a healthy and natural environment, a holistic lifestyle, adequate bodywork through Asanas, Mudras and Kriyas, invigorating breath work through the use of Pranayama and the production of healthy thought process through the higher practices of Jnna and Raja Yoga. The application of yoga theraphy can be correlated with the Pancha Koshas and various yoga practices may be used as therapeutic interventions at different levels in this respect.

- Annamaya Kosha (anatomical level): Jattis (simple units of movements), Mudras (gestures for energy generation and conservation), Kriyas (structured movements), Asanas (steady and comfortable postures) along with the dietary modifications and control.

- Pranamaya Kosha (physiological level): Shat Karmas (cleaning actions), various pranayamas, development of breath awareness, working on breath movement coordination and the energizing and balancing of the Pranic energy.

- Manomaya Kosha (psychological level): Trataka (concentrated gaze), Dharana (concentration), Dhyana (meditation), Japa and Japa-Ajapa practices are useful. Various aspects of concentration such as the Mandala Dharana and other Yoga Drishti techniques are available for this purpose.

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- Vijnanamaya Kosha (intellectual level): Swadhyaya (self analysis), Satsangha) lectures and spiritually uplifting exchange) along with the wonderful Jnana Yoga and Raja Yoga relaxation and concentration practices of yoga.

- Anandamaya Kosha (universal level): Learning to implement the pricipales of Karma Yoga (Yoga as skilled action performed without expectation) and following the principles of action in relaxation help us to bring about joy in all our activities. A realization that we live in a blissful universe and that all life is joy is to be brought about in this intervention through use of Bhakti Yoga, Karma yoga and other aspects like Bhajana, Yogic councelling and Satsangha.

Some physiological benefits of yoga

a. Stable autonomic nervous system equilibrium, with a tendency toward parasympathetic nervous system dominance rather than the usual stress-induced sympathetic nervous system dominance.
b. Pulse rate and blood pressure decreases. Respiratory rate decreases. EEG – alpha waves increase.
c. Theta, delta and beta waves also increase during various stages of meditation. Cardiovascular efficiency increases.
d. Respiratory efficiency, amplitude and smoothness increases, tidal volume, vital capacity and breath- holding times also increases.
e. Gastrointestinal function and endocrine function normalizes. Excretory functions improve.
f. Musculoskeletal flexibility and joint range of motion increase. Posture improves. Strength, resiliency endurance and energy level increase.
g. Weight normalizes and sleep improves. Immunity increases and pain decreases.

Some psychological benefits of yoga

a. Somatic and kinesthetic awareness increase.
b. Mood improves and subjective well being increases.
c. Shelf acceptance and shelf actualization increase.
d. Social adjustment increases. Anxiety, depression and hostility decreases.

Some biochemical effect of yoga

a. The biochemical profile improves, indicating an anti-stress and antioxidant effect which is important in the prevention of degenerative diseases.
e. Decreased level of blood glucose, total white blood cell count, total cholesterol, triglycerides, LDL and VLDL.
b. Increased levels of HDL cholesterol, ATPase, Hematocrit, hemoglobin, thyroxin, lymphocytes, vitamin C and total serum protein.

Conclusion

Yoga helps us regain the ease we had lost through dis-ease (as implied by sthira sukham asanam). It also produces mental equanimity (samatvam yoga uchyate) where the opposites cease to affect (tato dwandwa anabhigatha). This enables us to move from a state of illness and disease to one of health and well being that ultimate allows us to move from the lower animal nature to the higher human nature and finally highest Divine Nature that is our birthright.

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Physical activity and exercise in obese individuals

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Obesity is defined as the excessive buildup of fat in the adipose tissue of the body. An index commonly used to define such condition is BMI (body mass index), which is ≥30 kg/m2 in Caucasian population and ≥25 kg/m2 in Asian population. Obesity is believed to be a worldwide problem with consequences in healthiness and beyond. It is one of the major risk factors for several diseases including: hypertension; type 2 diabetes mellitus; dyslipidaemia; obstructive sleep apnea and sleep-disordered breathing; certain cancers; major cardiovascular diseases; impairment of physical fitness. Some obese individuals, especially severe patients, may exhibit: respiratory muscle weakness; metabolic abnormalities (blood sugar, leptin and insulin hormone regulations, and lipid metabolism [total cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein]); low-grade inflammation. In addition, they also present an alteration in the autonomic nervous system by causing chronic sympathetic overstimulation and increasing catecholamine levels, and mostly depressing vagal activity and reduced baroreflex sensitivity. Exercise here is defined as a division of physical activity that is structured, arranged and repetitive. It helps to promote and maintain fitness dimensions that consist of cardiopulmonary fitness; muscle strength; body composition; flexibility. It also counters the adverse effects of obesity such as cardiac autonomic function, prevents obesity-related diseases by improving weight control and metabolic profiles associated with obesity, and respiratory muscle strength. Nevertheless, the magnitude of its effect is governed by modes, the total dose and intensity of exercise intervention, individual variations, and concomitant weight loss produced by exercise. Dose refers to the total amount of energy expended in physical activity, whereas intensity reflects the rate of energy expenditure during such activity. The American College of Sports Medicine (ACSM) and the Center for Disease Control and Prevention (CDC) recommend that individuals should engage in 30 minutes or more of moderate-intensity physical activity on most, if not all, days of the week. Moderate-intensity activities are referred to those performed at a relative intensity of 40% to 60% of maximal oxygen consumption or 50 to 70% of his or her maximum heart rate (or absolute intensity of 4 to 6 metabolic equivalents). It is, thus, crucial for healthcare professionals to establish a well thought out and balanced physical activity programs for their patients as well as identify future research areas based on their experience.

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Origanal Article

Effects of Thai wand exercise on lung capacity in sedentary young adults

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Abstract

Sedentary lifestyle and physical inactivity in young adults have been associated with cardiovascular disease (CVD). Moreover, low cardiopulmonary fitness reveals high risk for all-cause mortality in sedentary individuals with or without underlying CVD. Thai wand exercise is a traditional exercise which believed to improve health-related quality of life and reduce cardiovascular risk factors. Therefore, the present study was aimed to determine the effect of Thai wand exercise on lung capacity and cardiovascular risk factors in sedentary young adults. Twenty nine sedentary young adults aged 18-25 years old in urban, Nakhon Pathom were recruited. All of them were performed 40 min/day, 3 days/week for 4 weeks of Thai wand exercise training. Anthropometry, body composition, blood pressure, chest expansion and lung capacity were measured before and after 4 weeks of training. Thai wand exercise significantly reduced waist circumference (71.5 ± 5.7, 70.4 ± 5.5 cm, $P < 0.01$), percentage of body fat (35.4 ± 4.0, 33.9 ± 3.3 %, $P < 0.01$) and fat mass (20.1 ± 4.0, 19.2 ± 3.6 kg, $P < 0.01$) and increased tidal volume (0.5 ± 0.2, 0.6 ± 0.1 L, $P < 0.01$), vital capacity (3.0 ± 0.6, 3.1 ± 0.7 L, $P < 0.05$), forced vital capacity (2.9 ± 0.7, 3.1 ± 0.7 L, $P < 0.01$) and chest expansion (upper; 3.2 ± 1.0, 3.9 ± 1.3 cm, $P < 0.01$), (middle; 3.7 ± 1.1, 4.4 ± 1.2 cm, $P < 0.01$), (lower; 3.8 ± 1.3, 4.7 ± 1.3 cm, $P < 0.01$). This study demonstrates that Thai wand exercise training improve lung capacity which related to physical health status. In addition, this training program reduced body and abdominal fat which related to risk factors for CVD. Therefore, Thai wand exercise is a good approach to improve health-related quality of life and reduce cardiovascular risk factors.

Keywords: *Thai wand exercise, lung capacity, sedentary*

Sedentary lifestyle and physical inactivity represent the high prevalence and public health concern in developed and developing countries. In Thailand, *21% and 24% of men and women* adolescence are lack of physical activity. Moreover, the prevalence of individuals with sedentary lifestyle and physical inactivity is rising steadily.[1] Sedentary lifestyle and physical inactivity in young adults have been associated with cardiovascular disease (CVD).[2] The association between sedentary lifestyle and the development of obesity and metabolic syndrome during adolescence is revealed.[3-4] Moreover, low cardiopulmonary fitness reveals high risk for all-cause mortality in sedentary individuals with or without underlying CVD.[5] Interestingly, sedentary lifestyle is associated with less efficient lung capacity.[6] Moreover, individuals with sedentary lifestyle are also associated with high prevalence of obesity, development of restrictive of lung diseases and cardiovascular mortality.[7] Involvement in certain physical activities and regular exercise could improve in respiratory muscle strengthening and lung capacity.[8-10]

To date, endurance exercise training has been recommended to promote health status and reduce body fat which is associated with a better health-related quality of life.[11-12] Nevertheless, normal fitness training in gyms is inconvenient and financial cost for young adults. Thus, the present study offers a traditional exercise which is called Thai wand exercise. Thai wand exercise is series of exercise which flexibility, balance, endurance and diaphragmatic breathing exercise is combined. All of movement during exercise is simple and smooth with full range of motion of shoulders and waist.[13] The movements combination with diaphragmatic breathing exercise could improve respiratory muscle strengthening and lung capacity and also reduce risks for CVD. The previous study demonstrated that older individuals who performed 40-minute Thai wand exercise per day, 3-5 days per week for 15 weeks revealed improvement of body flexibility, functional capacity, abdominal obesity and health-related quality of life.[13] However, no study has determined the effectiveness of Thai wand exercise on lung capacity and cardiovascular risk factors. Therefore, the present study is aimed to determine the effect of Thai wand exercise on lung capacity and cardiovascular risk factors in sedentary young adults.

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Materials and Methods

Subjects

Twenty nine subjects (4 men and 25 women) aged 18-25 years old were recruited from the general population in the urban area of Nakhon Pathom, Thailand by Global Physical Activity Questionnaire. Sedentary lifestyle and physical inactivity were defined as the number of hours per week spent during leisure time, e.g., computer use, video game playing, television viewing, and reading. Time spent was measured by a physical activity questionnaire. The questionnaire included information of activity types, frequencies and average of time spent on these activities

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over the last three months before the interviews. Subjects with underlying of CVD, hypertension, impaired mobility, hepatic and renal function were excluded. They performed Thai wand exercise for 4 weeks and no leisure-time physical activity, or having activities for less than 20 minutes or fewer than 3 times per week throughout the study (Figure 1). All parameters were collected before and after 4 weeks of training. This study was conducted according to the guidelines for Human Research Protection which was approved by the Ethical Committee of Mahidol University Institutional Review Board (MU-IRB 2010/294.2810). Written and verbal informed consents were obtained from all subjects. Verbal consent was witnessed and formally recorded.

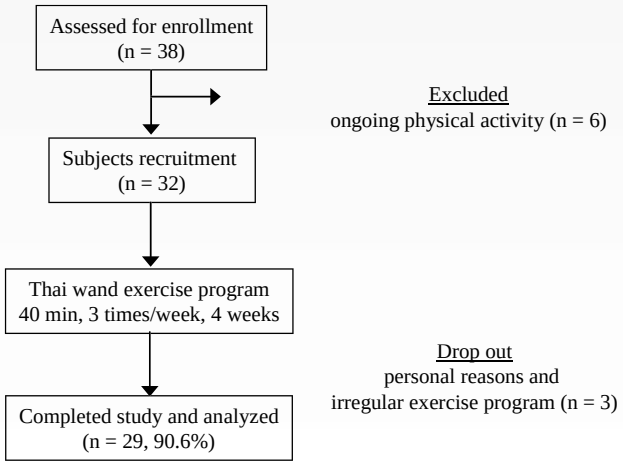

Figure 1. Flow chart of the study representing subject enrolment, exercise intervention and data analysis.

Anthropometric and body composition measurements

Height and body weight (BW) (with subjects in their minimal clothing) were measured with a laboratory scale (Detecto, Webb City, MO U.S.A.). Body mass index (BMI) was calculated as the ratio of BW in kilograms to height in meters squared. The body density was calculated based on skinfold thickness in the area of biceps, triceps, subscapular and suprailiac using a skinfold caliper (British Indicators Ltd, St Albans, Herts, England) and substitute the log of their sum into one of the equations by Durnin and Womersley.[14] Percentage of body fat (% BF) was calculated using the Siri equation as following: % BF = (495/Body Density) – 450.[15] Fat mass (FM) and fat free mass (FFM) were then calculated from % BF. The waist circumference (W) was measured midway between the costal margin and iliac crest. Hip circumference (H) was measured around buttock at the level of maximal dimension in a free-standing position.

Blood pressure measurements

Blood pressure (BP) and heart rate (HR) were measured after a 20-minute rest, using the automatic sphygmomanometer (Datascope ACCUTORR#1A, Japan) on the right upper arm in the sitting position. Average BP and HR were determined from three measurements after an almost stable BP seemed to have been reached. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were used to calculate mean arterial pressure (MAP) by using the formula: MAP = DBP + 1/3 (SBP – DBP)

Chest expansion measurements

Chest expansion was measured the circumferential change of the thorax at three levels (axillary, xiphisternal and the tenth costal cartilage) using tap. The difference between normal breath and deep breath was collected for chest expansion. Chest expansion was measured twice before and after 4 weeks of training and the best value was taken.

Pulmonary function test

Pulmonary function of all subjects was measured before and after 4 weeks of training using a gas analyzer system (Vmax encore 29, VIASYS Healthcare Inc., USA). The gas analyzer system was calibrated prior to each test and the tests were carried out according to standardized clinical spirometry procedure.[16-17] Subjects had to remain in the upright position with a tightly fitted nose clip during the test. The largest value obtained from three executions which did not differ by more than 5% or 100 milliliters was taken. The percents of predicted value were calculated using Thai pulmonary function predicted values available from Faculty of Medicine Siriraj Hospital, Mahidol University (2000).

Exercise intervention

Subjects completed a 4-week Thai wand exercise program which consisted of three 60-minute sessions each week at an intensity of 50% of their individual age-predicted maximum heart rate. The program was a 10-form Thai wand exercise modified by Puengsuwan and colleagues in 2008.[13] The program emphasized the range of motion of upper limbs and trunk for enhancing lung expansion and diaphragmatic breathing exercise was combined (1:2 inspiration to expiration ratio). Subjects were instructed to deeply inhale when hands were moving outwards and slowly exhale when hands were moving inwards. Diaphragmatic breathing exercise can improve ventilation, reduce work of breathing and dyspnea. All subjects were asked to reassess the breathing techniques every week for maintain the proper skills. The Thai wand movements were performed in a series, simple and smooth. In addition, breathing and mental concentration were naturally coordinated. In this study, subjects learned to perform Thai wand exercise on the first day of the intervention period. A video was also given to each subject to facilitate daily self-practice. They performed Thai wand exercise training at home according to a video recording for one session (40 minutes) per day, 3 days/week for 4 weeks. All subjects were asked to perform Thai wand exercise that led by a qualified instructor at laboratory every week for reassessment and adjustment of the program. Each subject was called every week to check their cooperation to the program. Subjects were asked to

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do leisure-time physical activity or activities for less than 20 minutes or fewer than 3 times per week though the experiment. Subjects were asked to record their physical activity for three days; two weekdays and one weekend day during one week before the start of the experiment and every week during the experiment.

Statistical analysis

All statistics were calculated by using the SPSS statistical software 18.0. Parametric tests were used to test a normal distribution of data. The normally distributed parameters were analyzed by paired *t*-test. Wilcoxon-signed rank test was used to compare non-normally distributed parameters. The data were expressed as mean ± SD (range: minimum and maximum values), mean differences and 95% confidence intervals. Significant differences were defined as $P < 0.05$.

Results

Thirty eight young adults were recruited in this study. Six young adults were excluded from participation because of ongoing physical activity. Thirty two sedentary young adults performed 40 minutes of Thai wand exercise training per day, 3 days/week for 4 weeks. During the training period, three subjects withdrew from participation because of personal reasons and irregular exercise program. After 4 weeks of training, 29 sedentary young adults (4 men and 25 women), aged 19 ± 0.9 years old, completed the study (90.6%). (Table 1)

Table 1. Physical characteristics and pulmonary function

	Sedentary young adults (n = 29)
Gender	4M, 25W
Age (years)	19.0 ± 0.9
BW (kg)	56.3 ± 7.2
BMI (kg/m^2)	21.8 ± 2.3
Height (cm)	106.7 ± 7.6
FVC (%pred)	98.5 ± 10.6
FEV_1 (%pred)	92.9 ± 12.1
FEV_1/FVC(%pred)	94.6 ± 9.1
FEF 25%-75% (%pred)	97.7 ± 21.0
PEF (%pred)	93.9 ± 18.1

Values are expressed as mean ± SD. BW, body weight; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; FEV_1/FVC, the ratio of forced expiratory volume in one second to forced vital capacity; FEF 25%-75%, Forced mid-expiratory flow; PEF, peak expiratory flow; % pred, percent of predicted value.

Changes in anthropometric and body composition

Combined data showed that Thai wand exercise training significantly reduced W -1.1 cm (95% CI: -1.8 to -0.4 cm, 71.5 ± 5.7, 70.4 ± 5.5 cm, $P < 0.01$), % BF -1.5% (95% CI: -2.3 to -0.6 %, 35.4 ± 4.0, 33.9 ± 3.3 %, $P < 0.01$) and FM -0.9 kg (95% CI: -1.4 to -0.3 kg, 20.1 ± 4.0, 19.2 ± 3.6 kg, $P < 0.01$) after 4 weeks of training (Table 2). However, there was no significant difference in BW, BMI, H, waist-to-hip ratio (W/H ratio), SBP, DBP, MAP and HR before and after 4 weeks of training (Table 2).

Table 2. Changes in physical characteristics before and after 4 weeks of training in sedentary young adults.

	Sedentary young adults (n = 29)			
	before	**after**	**Mean difference (95% CI)**	***P* value**
BW (kg)	56.3 ± 7.2 (44 – 80)	56.3 ± 7.5 (43.2 – 79)	-0.0 (-0.5 to 0.5)	0.95
BMI (kg/m^2)	21.8 ± 2.3 (18 – 26.6)	21.8 ± 2.4 (17.7 – 26.6)	-0.0 (-0.2 to 0.2)	0.87
% BF	35.4 ± 4.0 (24.6 – 41)	34.0 ± 3.3 (20.9 – 40)	-1.5 (-2.3 to -0.6)	<0.01**
FM (kg)	20.1 ± 4.0 (13.1 – 31)	19.2 ± 3.6 (13 – 28)	-0.9 (-1.4 to -0.3)	<0.01**
FFM (kg)	36.3 ± 4.3 (29.5 – 49)	36.3 ± 4.6 (29.9 – 48)	-0.0 (-0.5 to 0.5)	0.95
W (cm)	71.5 ± 5.7 (60 – 89.5)	70.4 ± 5.5 (59 – 85)	-1.1 (-1.8 to -0.4)	<0.01**
H (cm)	94.6 ± 5.7 (83.5 – 106)	94.4 ± 5.6 (83 – 105)	-0.2 (-1.9 to 1.5)	0.50
W/H ratio	0.75 ± 0.05 (0.7 – 0.9)	0.75 ± 0.04 (0.7 – 0.9)	0.0 (-0.1 to 0.1)	0.51
SBP (mmHg)	105.1 ± 11.4 (84 – 134)	104.4 ± 10.9 (80 – 126)	-0.7 (-4.4 to 3.1)	0.73
DBP (mmHg)	64.0 ± 8.7 (52 – 92)	63.2 ± 7.4 (49 – 83)	-0.8 (-3.7 to 2.1)	0.57
MAP (mmHg)	77.7 ± 8.3 (63.7 – 97.3)	76.9 ± 7.9 (59.3 – 95.3)	-0.8 (-3.2 to 1.6)	0.51
HR (bpm)	80.6 ± 9.7 (66 – 106)	79.5 ± 10.0 (68 – 111)	-1.1 (-5.5 to 3.4)	0.62

Values are expressed as mean ± SD (range: minimum and maximum values). BW, body weight; BMI, body mass index; % BF, percentage of body fat; FM, fat mass; FFM, fat free mass; W, waist circumference; H, hip circumference; W/H ratio, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic; MAP, mean arterial pressure; HR, heart rate.
*P value < 0.05, ** P value < 0.01; paired *t*-test.

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Changes in pulmonary function and chest expansion

After 4 weeks Thai wand exercise training, tidal volume (TV) 0.1 L (95% CI: 0.1 to 0.1 L, 0.5 ± 0.2, 0.6 ± 0.1 L, $P < 0.01$), vital capacity (VC) 0.1 L (95% CI: 0.0 to 0.2 L, 3.0 ± 0.6, 3.1 ± 0.7 L, $P < 0.05$) (Table 3 and Figure 2) and forced vital capacity (FVC) 0.1 L (95% CI: -0.0 to 0.3 L, 2.9 ± 0.7, 3.1 ± 0.7 L, $P < 0.01$) (Table 3 and Figure 3) were significantly increased and the ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) -4.9% (95% CI: -9.3 to -0.4%, 94.6 ± 9.1, 89.8 ± 7.6%, $P < 0.05$) (Table 3 and Figure 3) was significantly decreased. However, there was no significant difference in maximal voluntary ventilation (MVV) and FEV_1 before and after 4 weeks of training (Table 3). Combined data showed that Thai wand exercise training significantly increased chest expansion: upper chest 0.6 cm (95% CI: 0.3 to 0.9 cm, 3.2 ± 1.0, 3.9 ± 1.3 cm, $P < 0.01$), middle chest 0.7 cm (95% CI: 0.4 to 1.0 cm, 3.7 ± 1.1, 4.4 ± 1.2 cm, $P < 0.01$) and lower chest 0.9 cm (95% CI: 0.5 to 1.3 cm, 3.8 ± 1.3, 4.7 ± 1.3 cm, $P < 0.01$) after 4 weeks of training (Table 3).

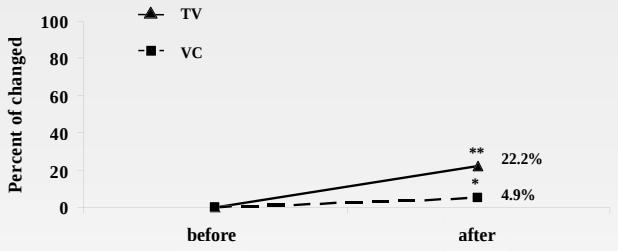

Figure 2. Percent change of tidal volume and vital capacity before and after 4 weeks of training in sedentary young adults (n = 29). Values are expressed as percent change. TV, tidal volume; VC, vital capacity; $^{*}P$ value < 0.05, $^{**}P$ value < 0.01.

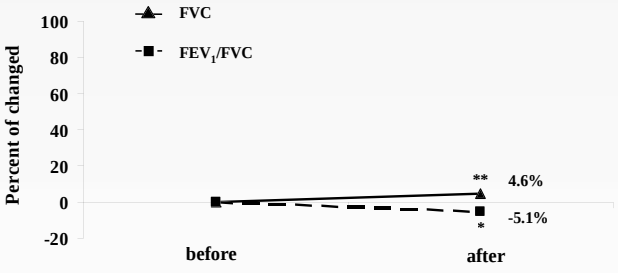

Figure 3. Percent change in FVC and FEV_1/FVC before and after 4 weeks of training in sedentary young adults (n = 29). Values are expressed as percent change. FVC, forced vital capacity; FEV_1/FVC, the ratio of forced expiratory volume in one second to forced vital capacity; $^{*}P$ value < 0.05, $^{**}P$ value < 0.01.

Table 3. Changes in pulmonary function parameters before and after 4 weeks of training in sedentary young adults

	Sedentary young adults (n = 29)			
	before	**after**	**Mean difference (95% CI)**	***P* value**
TV (L)	0.5 ± 0.2 (0.3 – 0.9)	0.6 ± 0.1 (0.5 – 1.0)	0.1 (0.1 to 0.1)	<0.01**, a
VC (L)	3.0 ± 0.6 (2.3 – 5.1)	3.1 ± 0.7 (2.3 – 5.3)	0.1 (0.0 to 0.2)	<0.05*, a
MVV (L)	106.7 ± 32.0 (59 – 194)	109.6 ± 32.0 (56 – 173)	2.9 (-2.2 to 8.1)	0.25, a
FVC (L)	2.9 ± 0.7 (1.8 – 4.9)	3.1 ± 0.7 (2.2 – 4.8)	0.1 (-0.0 to 0.3)	<0.01**, b
FEV_1 (L)	2.8 ± 0.7 (2.0 – 4.9)	2.8 ± 0.6 (2.0 – 4.8)	-0.1 (-0.2 to 0.0)	0.11, b
FEV_1/FVC	94.6 ± 9.1 (73.1 – 119.1)	89.8 ± 7.6 (70.9 – 100)	-4.9 (-9.3 to -0.4)	<0.05*, a
Upper chest (cm)	3.2 ± 1.0 (1.5 – 4.5)	3.9 ± 1.3 (1.5 – 6)	0.6 (0.3 to 0.9)	<0.01**, a
Middle chest (cm)	3.7 ± 1.1(2 – 5)	4.4 ± 1.2 (2.5 – 6)	0.7 (0.4 to 1.0)	<0.01**, a
Lower chest (cm)	3.8 ± 1.3 (2 – 6)	4.7 ± 1.3 (2 – 7)	0.9 (0.5 to 1.3)	<0.01**, a

Values are expressed as means ± SD (range: minimum and maximum values). TV, tidal volume; VC, vital capacity; MVV, maximal voluntary ventilation; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; FEV_1/FVC, the ratio of forced expiratory volume in one second to forced vital capacity.
* P value < 0.05, ** P value < 0.01; ^{a}paired t- test, ^{b}Wilcoxon-signed rank test.

Discussion

This is the first study in sedentary young adults who performed 40 min/day, 3 days/week for 4 weeks of Thai wand exercise training. Results showed significant improvement in lung capacity, chest expansion, body and abdominal fat.

Pulmonary function test is used as a tool in general health assessment in healthy subjects and patients.[16-17] These parameters are a long-term predictor for overall survival rates in patients with chronic lung disease.[18] Interestingly, sedentary lifestyle is associated with less efficient lung capacity which leads to restrictive of lung diseases.[7] Moreover, individuals with sedentary lifestyle are also associated with high prevalence of obesity and cardiovascular mortality.[6-7]

This study found that after 4 weeks of Thai wand exercise training with diaphragmatic breathing exercise chest expansion, TV, VC, FVC and FEV_1/FVC were improveed in sedentary young adults. The findings on chest expansion and lung capacity are supported by several previous studies.[13, 19-22] The movements of Thai wand exercise are similar to chest wall mobilization which includes stretching of intercostal muscles, extension and lateral flexion of trunk and rotation of vertebral segments.[20-21] The increase in chest expansion was demonstrated in patients with chronic lung disease who performed self-stretching techniques. Moreover, the improvement in TV, VC, FVC and FEV_1/FVC following Thai wand exercise training with diaphragmatic breathing exercise may be achieved by increasing inspiratory force and lengthening of intercostals and accessory muscles.[20, 23-24] Thus, effectiveness of Thai wand exercise is also interesting because the results suggest benefits in patients with tight chest wall and obstructive of lung diseases.

In this study, sedentary young adults who performed 40-minute Thai wand exercise training per day, 3 days/ week for 4 weeks showed significant improvement in body and abdominal fat. It has been known that body and abdominal fats are strong independent predictors of cardiovascular incidents and influence to numerous risk factors for CVD were reduced.[25-26] A previous study reported that abdominal fat was reduced due to the movement of Thai wand exercise which occurs around waist. The consequence of abdominal and back muscles contraction and strengthening may contribute to the reduction of waist circumference.[13] Moreover, the reduction abdominal fat response to the exercise training and greater energy expenditure in young and elderly was demonstrated.[27-28]

The limitation of this study is its short duration. A previous study of a 12-week yoga program has shown improvement in FEV_1 and peak expiratory flow in yogis, marathon runners and sedentary workers.[29] Although, changes may have been apparent, an extension in the duration of the study has been performed. Most subjects in this study (90.6%) continue practicing Thai wand exercise with high compliance rate of daily self-practice. Therefore, future studies should consider long term effects of the exercise program to assess the long term adherence of participants. In addition, due to the absence of control group in this study, future studies should perform to decrease the errors of the results.

Conclusion

This study demonstrates that Thai wand exercise training improve lung capacity which is related to physical health status. In addition, this training program reduced body and abdominal fat which is related to risk factors for CVD. Therefore, Thai wand exercise is a good approach to improve health-related quality of life and may reduce cardiovascular risk factors.

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Conflict of Interest: None to declare

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Origanal Article

Effect of melatonin on hippocampal CA3 pyramidal cells following dexamethasone treatment in mice

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Abstract

Prolonged exposure to dexamethasone (DEX), a synthetic glucocorticoid receptor agonist, has been shown to induce neuronal cell death in the hippocampus. Cell death is particularly pronounced in the CA3 pyramidal neurons, inducing cognitive dysfunction. The aim of this study was to investigate the effect of melatonin on hippocampal CA3 pyramidal cells in dexamethasone treated mice. The mice received a daily injection (i.p.) of dexamethasone at 60 mg/kg for 21 days. Melatonin (10 mg/kg) was injected (i.p.) before dexamethasone treatment for 30 minutes. Hippocampal CA3 damage was investigated by 0.1% cresyl violet staining. The co-treatment with melatonin 30 min prior to dexamethasone was found to significantly protect against the morphological changes and neuronal loss in the CA3 region. These results suggest that melatonin may have a protective effect against neuronal cell damage following dexamethasone treatment, and this may be helpful for improving brain cognitive function.

Keywords: *stress, hippocampus, CA3 pyramidal cell, dexamethasone, melatonin*

Stressful conditions can precipitate anxiety and depression, which can lead to excessive production of free radicals which in turn results in oxidative stress. Stressful events also induce the release of adrenal stress hormones, including catecholamines and glucocorticoids.[1-3] Chronic stress or prolonged exposure to high levels of corticosterone induces neuropathological alterations, such as dendritic atrophy in the hippocampal or striatal neurons.[4,5] Similarly, administration of dexamethasone (DEX, a glucocorticoid receptor agonist) mimics the increased plasma level of corticosterone and harmful effects of DEX have been reported after the acute or prolonged administration of this drug.[1-3] Moreover, administration of DEX has been shown to produceneuronal cell death in CA3 and dentate gyrus of the hippocampus.[6] Dexamethasone has been reported to endanger hippocampal neurons by exacerbating the excitotoxic glutamate-calcium-reactive oxygen species (ROS) cascade.[7,8] Recently, melatonin has been proposed to have antioxidant activity and anti-inflammatory effects against various pathophysiologic mechanisms.[9,10] This compound has been found to prevent methylphenyltetrahydro-pyridine (MPTP) induced cell damage to the substantia nigra in experimental Parkinsonism, thereby preventing disease progression in the animals.[11,12] Melatonin pretreatment reduced cerebral infarct size and edema after middle cerebral artery occlusion and ischemia-reperfusion injury in rats.[13] Furthermore, many studies examining the neuroprotective effects of melatonin in various regions of the central nervous system have been demonstrated.[14]

The aim of this study was to investigate the effect of melatonin treatment on hippocampal CA3 cells following dexamethasone treatment in mice.

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Materials and methods

Animals

Adult male ICR mice (25-30 grams) obtained from the National Laboratory Animal Center, Mahidol University Salaya Campus were used in this study. The experimental protocols conformed to the Home Office (UK) Animal Scientific Procedure Act 1986, and were approved by the animal care and use committee of Mahasarahkarm University. Mice were housed one per plastic cage in a room. Animals were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature (24 ± 2°C) room, with free access to food (normal rodent food) and water.

Chemicals

Dexamethasone was purchased from T.P. Drug Labolatories Co.,Ltd., Thailand and melatonin was purchased from Sigma Chemical Co., St. Louis, Mo.

Drug preparation

Melatonin was freshly prepared before injection. Melatonin (10 mg/kg)[15] was dissolved in a small volume of ethanol and then diluted with saline. Final concentration of ethanol was 5%. Melatonin solution tubes were wrapped in aluminum foil to prevent light-induced degradation.

Experimental design

Mice were randomly divided into four groups (6 animals/ group). The first group (control group), mice were i.p. injected with vehicle followed by 0.9% NSS 1

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ml/kg. The second group (DEX group), mice were treated with vehicle followed by dexamethasone 60 mg/kg (i.p.).[16] The third group (Mel group), mice were injected with melatonin (10 mg/kg) followed by 0.9% NSS 1 ml/kg (i.p.). The fourth group (Mel+DEX group), mice were injected with melatonin (10 mg/kg) followed by dexamethasone 60 mg/kg (i.p.). Vehicle (5% ethanol in 0.9% NSS) or melatonin was administered 30 min before the 0.9% NSS or dexamethasone treatment. Mice were injected with vehicle, melatonin and dexamethasone once daily between 5.00 pm and 6.00 pm for 21 days.

Histological study

After 21 days treatment, mice were deeply anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4), and followed by 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and post-fixed with the same fixative overnight at 4°C. Following cryoprotection in 30% sucrose in 0.1 M PBS overnight at 4°C, free-floating coronal sections at the level of the dorsal hippocampus were cut at 25 μm thickness on a cryostat microtome according to the stereotaxic atlas of the mouse brain.[17] These were stored in 0.1 M PBS at 4°C prior to 0.1% Cresyl violet staining.

Hippocampal CA3 pyramidal cell counts and thickness were analyzed using systematic random sampling techniques. All microscopic analyses were conducted on a digital Axio Cam ICC3 analysis system (Carl Zeiss, Germany) at 20× magnification and the numbers of neurons were counted (UTHSCA Image Tool for Windows version 3.0 software)

Data analysis

Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range tests. Results are presented as means±S.E.M. A level of $P < 0.05$ was accepted as statistically significant.

Results

Effects of melatonin on CA3 thickness

After treatment for 21 days, the thickness of the CA3 pyramidal cell layer in the DEX group was significantly less than the control and Mel group (P<0.05). In melatonin pretreated mice (Mel+DEX group), the neurons of the hippocampal CA 3 regions were significantly preserved compared to mice receiving dexamethasone treatment only (P<0.05) (Figure 1).

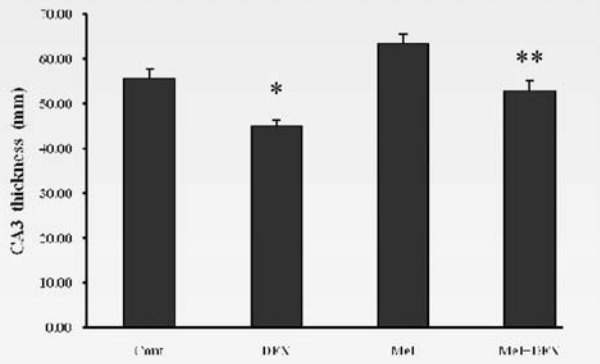

Figure 1. Quantitative analysis of CA3 pyramidal layer thickness. Values are expressed as means±SEM per section. The CA3 pyramidal layer thickness in the dexamethasone treated group was significantly less than control group (P<0.05) and the CA3 pyramidal layer thickness in the Mel+DEX group was significantly more than DEX group (P<0.05). Six mice were used for each treatment group. *P<0.05, compared with control group; **P<0.05, compared with dexamethasone treated group.

CA3 cell number

Quantitative analysis of the number of CA3 hippocampal neurons in the DEX group indicate significantly lower number than in the control group (P<0.05). Furthermore, the number of CA3 hippocampal neurons in the Mel+DEX group was significantly higher than the dexamethasone treated group (P<0.05). This data revealed that melatonin attenuated dexamethasone-induced neuronal loss in the CA3 cells layer (Figures 2 and, 3).

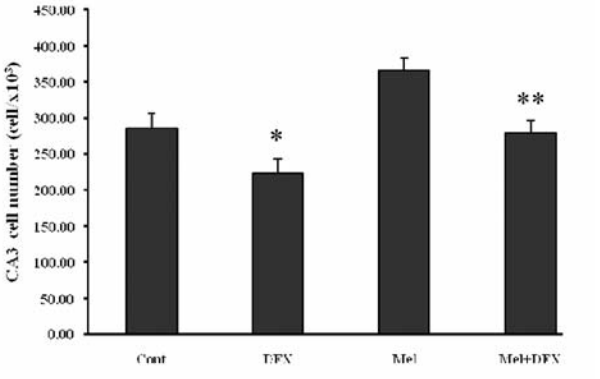

Figure 2. Quantitative analysis number of neuronal cells in CA3 hippocampal subfield. Values are expressed as means±SEM per section. Number of CA3 neuronal cells in the dexamethasone treated group was significantly less than control group (P<0.05) and the number of CA3 neuronal cells in the melatonin-pretreated groups was significantly more than dexamethasone-treated group (P<0.05). Six mice were used for each treatment group. *P<0.05, compared with control group; **P<0.05, compared with dexamethasone treated group

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Original Article

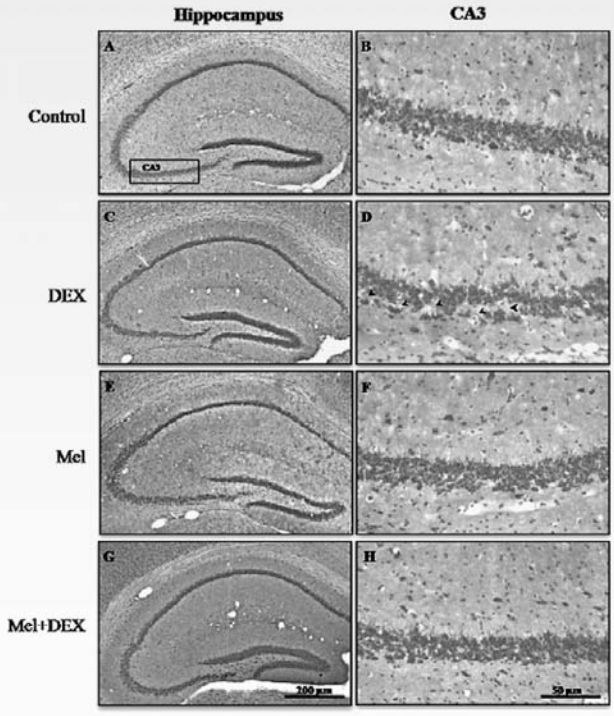

Figure 3. Photomicrographs of 0.1% cresyl violet staining in the hippocampus CA3 region are shown for (A-B): control (Cont.) group; (C-D): dexamethasone (DEX) treated group; (E-F): melatonin (Mel) treated group and (G-H): melatonin-pretreated (Mel+DEX) group. Morphological changes in pyramidal neurons were observed in CA3 in DEX treated mice (arrows). Scale bar = 200 μm in panels A, C, E and G. Scale bar = 50 μm in panels B, D, F and H.

Discussion

Elevated circulating adrenal steroids secreted during stressful situations may be involved in triggering morphological alterations in the brain. Repeated restraint stress or daily treatment with corticosterone induced a reversible atrophy and loss of hippocampal CA3 pyramidal neurons in rats.[18,19] A prenatal treatment with high doses of dexamethasone in rhesus monkeys resulted in an approximately 30% reduction of hippocampal size later in life.[20] Moreover, the influence of activated glucocorticoids on oxidative stress-induced neuronal cell death in vitro has been investigated by employing hippocampal model systems.[21] The effects of both dexamethasone and stress-induced glucocorticoids on hippocampus damage and memory dysfunction have also been reported. [22,23] Additionally, dexamethasone has been suggested to endanger hippocampal neurons by exacerbating the excitotoxic glutamate-calcium-reactive oxygen species (ROS) cascade.[7,8] The present study demonstrated that subchronic dexamethasone treatment caused hippocampus morphological change and a decrease in CA3 pyramidal neurons. These effects may be due to oxidative stress enhancement. Additionally, melatonin pretreatment prevented impairment of neurons in the CA3 region induced by dexamethasone. In this case, melatonin administration has been shown to be effective in counteracting the oxidative stress conditions induced by dexamethasone. This is likely to be due to melatonin has a remarkable anti-oxidant activity and plays a crucial role in the genesis of neurodegenerative diseases. In addition to scavenging oxygen free radicals like super oxide radical ($O2^{-}$), hydroxyl radical ($^{*}OH$), peroxyl radical (LOO^{*}) and peroxynitrite anion ($ONOO^{-}$), melatonin enhances the antioxidative potential of the cell by stimulating the synthesis of antioxidative enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX), and also the enzymes that are involved in the synthesis of glutathione.[24] A disturbance of melatonin rhythm and secretion has also been noted in patients suffering from certain neurodegenerative diseases.[25] Taken together, these finding indicated that melatonin has a neuroprotective role. However, the precise mechanism by which melatonin prevents dexamethasone-induced impairment of CA3 neurons needs to be further investigated.

Conclusion

In our study, melatonin was found to prevent neuronal loss from dexamethasone treatment in adult hippocampus CA3 regions. Therefore, melatonin may be helpful for ameliorate CA3 neuronal impairment caused by dexamethasone treatment which may be useful for preventing impairment of brain cognitive function.

Acknowledgments

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Conflict of Interest: None to declare

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Origanal Article

Protective effect of Shallot (*Allium ascalonicum*) on paracetamol-induced hepatotoxicity in mice

Watchara Kourjampa, Ampa Luangpirom

Abstract

Overdose of paracetamol causes hepatotoxicity by its toxic oxidation metabolites. Shallot (*Allium ascalonicum*) is rich in phenolic compounds and showed high antioxidant properties. An objective of this study is to evaluate the protective effect of *A. ascalonicum* against hepatotoxicity induced by paracetamol. Thirty male mice strain IRC were allocated into 5 groups, 6 mice for each, group I received distilled water 0.5 ml/100 gBW as negative control, group II received paracetamol 0.5 g/100 gBW as positive control, group III received *A. ascalonicum* juice at dose of 1.0 g./100 gBW as extract toxicity test, groups IV and V received *A. ascalonicum* juice at dose of 0.5 and 1.0 g/100 gBW, respectively for 4 days and co-treated with paracetamol 0.5 g/100 gBW for 14 days as treated groups. All groups were determined serum alanine aminotransferase (ALT) and examined histological changes of liver. Serum ALT of paracetamol treated group was increased in 3 times than those of the control group and was supported by high incidence of liver lesion. Both groups pretreated with *A. ascalonicum* juice before co-treated with paracetamol showed decreasing of serum ALT and percentage of liver lesion. From this study, it could be suggested that *A. ascalonicum* juice may be used for attenuation the hepatotoxicity induced by paracetamol.

Keywords*: paracetamol, Allium ascalonocum, hepatotoxicity, protective effect*

Paracetamol (acetaminophen) is safe at therapeutic dose, but causes liver failure in overdose by its toxic oxidation metabolites.[9] It was reported that oxidative stress constitutes a major mechanism underlying the pathogenesis of paracetamol induced liver damage.[1]

Shallot (*Allium ascalonicum*) is rich in phenolic compounds than other onion (*Allium cepa*). Moreover, it is a major source of quercetin, a flavonol used as a nutritional supplement for its anti-inflammatory and antioxidant properties.[14] Other herbs such as *Hibiscus sabdariffa* Linn. (rosselle) and *Camellia sinonsis* (green tea) contained high phenolic compounds.[2,13] They also revealed protective effect against paracetamol induced hepatotoxicity in mice.[9,6,10] This investigation was to evaluate the protective effect of *A. ascalonicum* juice against paracetamol induced hepatotoxicity in mice, which was induced by oral administration with paracetamol at dose of 50 mg/100 gBW for 14 days. The protective effect of *A. ascalonicum* juice was assessed by serum alanine aminotransferase (ALT) determination and liver histological studies. ALT is mostly found in hepatocytes and serum ALT is a biochemical marker for early acute hepatic damage.[3]

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Materials and Methods

Preparation of *A. ascalonicum* juice

Fresh bulbs of *A. ascalonicum* were purchased from grocery in Khon Kaen province, Thailand. They were extracted by juice extractor and diluted with distilled water at dose of 1.0 and 2.0 g/ml for oral administration (0.5 ml/100 gBW of mice) (as described by Ola-Madithir et al., 2008).[11]

Animals

Adult male mice (aged 8 weeks old) weighing 35-40 g were obtained from the National Laboratory Animal Center of Mahidol University, Salaya, Nakornprathom Province, Thailand. They were housed under a 12:12 of light-day cycle and at 25 ± 1°C, were fed with standard pellet diet and were provided with water *ad libitum*. The experimental protocols had been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2196)

Experiment

Thirty male mice were distributed into 5 groups of six animals each. Group I received distilled water 0.5 ml/100 gBW for 18 days as negative control, group II received distilled water 0.5 ml/100 gBW for 4 days and followed by treatment with paracetamol at dose of 50 mg/100 gBW for 14 days as positive control (as method of Luangpirom and Maynoi, 2007),[6] group III received *A. ascalonicum* juice 1 g/100 gBW for 18 days as extract toxicity test, groups IV and V were pretreated with *A. ascalonicum* juice at doses of 0.5 and 1.0 g/100 gBW, respectively for 4 days and then co-treated with paracetamol for 14 days as treated

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groups(as method of Ola-Madathir et al, 2008).[11] All groups were gavaged daily.

Biochemical test

At the end of experiment, blood samples were collected by cardiac puncture under ether anesthesia and serum alanine aminitransferase (ALT) was determined.

Liver histological studies

After blood sampling, liver were removed and preserved in Bouin's solution, followed by paraffin method process, sectioned 5 μm in thickness and the liver sections were stained with Haematoxylin and Eosin (H&E). The histological changes of liver were observed under light microscope and percentages of abnormal occurrence were compared among groups.

Statistical analysis

Data of serum ALT and percentage of hepatic lesion were expressed as the mean ± SD and were assessed by using One way analysis of variance (One way ANOVA). Duncan's multiple test was used to determine the difference among groups. Difference were considered to be statistically significant if $P < 0.05$.

Results

Alanine aminotransferase (ALT) determination

There was no difference of serum ALT level of treated group with *A. ascalonicum* (21.1±1.76 IU/L) as compared with the control (20.5 ± 1.58 IU/L). While the serum ALT of paracetamol treated group was significantly increased (66.5 ± 1.76 IU/L) which was 3 times of the control. Interestingly, the groups were pretreated with *A. ascalonicum* juice at doses of 0.5 and 1.0 g/100 gBW before co-treated with paracetamol revealed the significant decrease of serum ALT when compared to the paracetamol treated group ($P < 0.05$) (Figure 1).

Liver histological examination

Histology of liver in the paracetamol-treated group found high occurrence of liver lesion including lymphocytic infiltration (29.0 ± 4.63 %), necrosis area (39.0 ± 8.37 %) and microvesicular steatosis (31.84 ± 5.83 %) which was significantly differed from the control ($P < 0.05$). In the control and group treated with *A. ascalonicum* juice showed no different results. Interestingly, groups were pretreated with *A. ascalonicum* juice at doses of 0.5 and 1.0 g/100 gBW for 4 days and following by co-treated with paracetamol for 14 days were found the significant reduction of percentage of liver lesion when compared to the control ($P < 0.05$) (Table 1 and Figure 2).

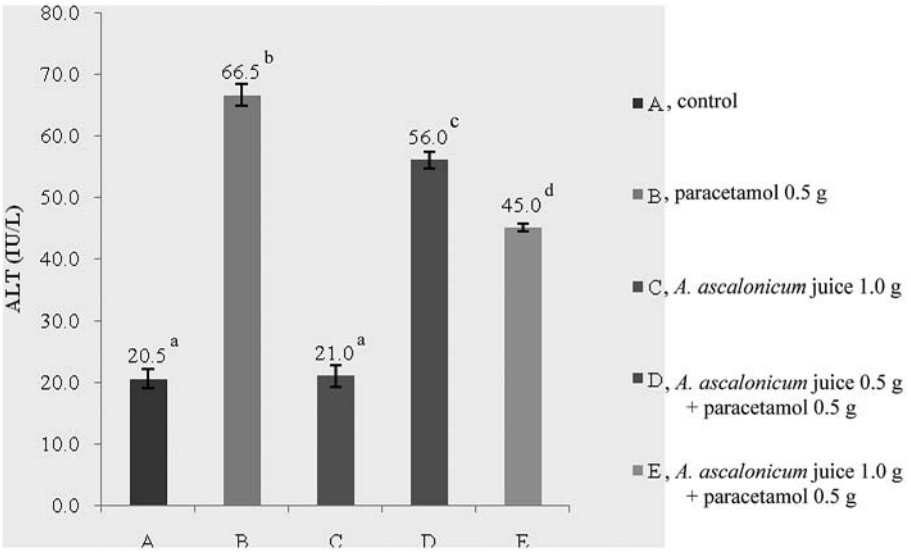

Figure 1. Serum ALT of mice treated with paracetamol and *A. ascalonicum* juice (same alphabet = not significantly different, $P > 0.05$; different alphabet = significantly different, $P < 0.05$).

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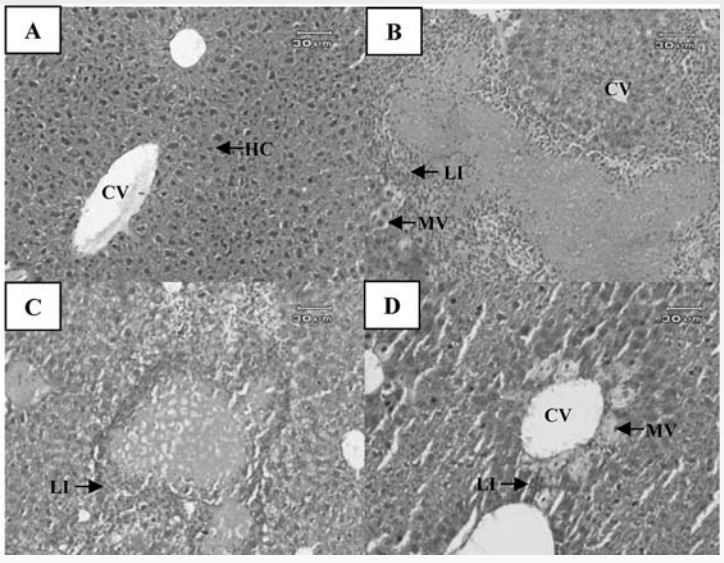

Figure 2. Liver paraffin section (H & E, bar = 30 μm): A, control group, showing normal architecture of hepatocyte (HC); B, paraetamol 0.5 g/100 gBW, showing hepatic lesion including lymphocytic infiltration (LI) and microvesicular steatosis (MV); C, paracetamol + *A. ascalonicum* juice 0.5 g/100 gBW, showing lymphocytic infiltration (LI); D, paracetamol + *A. ascalonicum* juice 1.0 g/100 gBW, showing lymphocytic infiltration (LI) and microvesicular steatosis (MV); CV = central vein.

Table 1. Hepatic lesion in mice treated with paracetamol and *A. ascalonicum* juice.

Treated group (g/100 gBW, N=6)	Microvesicular steatosis (X±SD, %)	Necrosis area (X±SD, %)	Lymphocytic infiltration (X±SD, %)	Total percentage of hepatic lesion
0	3.26±0.97 ^{a}	1.80±1.32 ^{a}	0.83±1.30 ^{a}	5.83 ^{a}
Paracetamol 0.5	31.84±5.83 ^{b}	39.00±8.37 ^{b}	29±4.63 ^{b}	99.84 ^{b}
A. ascalonicum juice 1.0	2.91±1.28 ^{a}	1.63±1.30 ^{a}	1.01±0.91 ^{a}	5.55 ^{a}
Paracetamol 0.5+juice 0.5	22.98±3.84 ^{c}	28.12±9.43 ^{c}	17.80±6.45 ^{c}	68.90 ^{c}
Paracetamol 0.5+juice 1.0	18.43±2.05 ^{d}	16.80±12.76 ^{d}	13.00±3.84 ^{d}	48.20 ^{d}

N = number of experimental animals
same alphabet = not significantly different ($P > 0.05$)
different alphabet = significantly different ($P < 0.05$)

Discussion

Serum ALT of the control group = 20.5 IU/L, while paracetamol treated group was increase in 3 times than those of the control. It was a biological marker of liver injury[3] and was supported by 99.84 % of liver lesion including microvesicular steatosis, necrosis area and lymphatic infiltration. Microvesicular steatosis, in which small droplets are present within the hepatocytes causing by abnormal cellular lipid peroxidation which can be induced by many drugs including paracetamol.[1] Lymphocytic infiltration is an incidence of acute liver inflammation.[5]

Interestingly, our biochemical and histological results showed that pretreatment with *A. ascalonicum* juice effectively reduced paracetamol induced hepatotoxicity by decreasing of serum ALT and percentage of hepatic lesion. The previous studies were reported that *A. ascalonicum* contained numerous natural phenolic compounds such as quercetin and flavonoids, especially quercetin which is a strong antioxidant.[8] They were capable to reduce level of reactive oxygen species(ROS) and to inhibit oxidation of low density lipoprotein (LDL) in serum of stretozotocin induced diabetic rats,[4] revealed protective effect on cadmium induced testicular oxidation damage in rats.[11]

In conclusion, our studies suggests that *A. ascalonicum* juice may use for attenuation the hepatotoxicity induced by paracetamol.

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Conflict of Interest: None to declare

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Origanal Article

Obesity and Respiratory Muscle Strength in Thai women

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Obesity is now well-known as an important risk factor for developing several respiratory diseases. Whether it is associated with respiratory muscle strength (RMS) impairment is unsettled. We aimed to investigate the influence of obesity on the RMS in Thai women aged between 20-60 years old. Sixteen obese (body mass index, BMI ≥ 25 kg/m^2) and 16 normal-weight women (BMI ≥ 18.5 to 22.9 kg/m^2) matched for age were studied. RMS including PImaxRV, PImaxFRC, Pnsn and PEmax were measured using a MICRORPM®, Medical, UK. A pneumotach airflow transducer (medium flow), BIOPAC Systems (USA) was used to measure tidal volume (VT), respiratory rate (RR) and total expired ventilation ($\dot{V}$E). BMI were 32.9±4.5 and 20.1±1.1 kg/m^2 whereas ages were 32.0±8.6 and 32.2±8.7 yrs in obese and lean groups, respectively. Absolute PImaxRV, PImaxFRC, Pnsn, PEmax and RMS in obese women were significantly higher than in normal-weight women ($p<0.001$). VT but not RR or $\dot{V}$E in obese women was significantly higher ($p<0.01$) than that of normal-weight peers. This study indicates that obese women studied have greater respiratory muscle strength than normal-weight peers. This could be due to deep breathing constantly loaded to the respiratory muscles and adaptation of muscle fibers.

***Keywords**: obesity, respiratory muscle strength, total expired ventilation*

Introduction

Obesity is worldwide epidemic with at least 300 million of them clinically obese.[1] An index that has been commonly used to define such condition is body mass index (BMI) which is ≥30 kg/m^2 in Caucasian population and ≥25 kg/m^2 in Asian population.[2] The second report on National Health Examination Survey of Thailand reported that 19.2% of men and 33.9% of women had BMI between 25-30 kg/m^2, whereas 3.5% of men and 8.8% of women had BMI >30 kg/m^2.[3] In 2010, we reported the prevalence of obesity and overweight 12 to 18 year-old-adolescents of 4.9% using the sex-specific BMI-for-age and 13.7% using the Thai standard W/H.[4]

Obesity has been demonstrated to be related to risk factors of many diseases such as cardiovascular disease, myocardial infarction, diabetes mellitus, systemic arterial hypertension, pulmonary dysfunction and respiratory failure.[1,5,6,7,8] Studies on respiratory muscle strength (RMS) in obese population report conflicting results. Maximum respiratory pressures in obese individuals are often normal.[9,10,11,12,13] On the other hand, some obese individuals, especially severe patients, have lower maximum respiratory pressures[14,15,16,17,18] or show higher RMS which might have occurred due to a probable adaption of muscle fibers.[19] Interestingly, several studies reported that inspiratory muscle training (deep breathing) in mild to moderate chronic obstructive pulmonary disease patients could improve respiratory muscle performance (tidal volume, VT, was increasing) indicating that respiratory muscle changes in strength or endurance.[10,20,21,22,23] We therefore aimed to investigate the strength of respiratory muscles, VT and $\dot{V}$E in obese Thai women.

Materials and Methods

Study designs

The designs of this study were descriptive and analytical. We divided women aged between 20-60 years into 2 groups: 16 obese (BMI≥25 kg/m^2) and 16 lean (BMI≥18.5 to 22.9 kg/m^2) participants.

Study population

All participants had FEV1>80% (only one normal-weight woman had FEV1=77%). Each obese woman's age was matched with a normal-weight peer of the same age. To avoid possible factors affecting respiratory muscle strength test, subjects with smoking history, sign of bronchiectasis, angina pectoris, hypertension, restrictive pulmonary disease, chronic obstructive pulmonary disease, obstructive sleep apnea, neuromuscular diseases and history of chronic steroid use were not included in this study.

Ethical approval

A written informed consent from the participant was obtained before testing. The methods of this study was reviewed and approved by the Khon Kaen University Ethics Committee for Human Research.

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Experiment protocol

Body mass index (BMI), hip and waist circumferences

Height, weight, hip and waist circumference were measured according to the WHO guidelines[24]. Participants wore light clothing and no shoes. Weight was determined using a digital scale (TCS-150-B), to the nearest tenth. Height was measured standing with feet together and arms relaxed at the sides. The BMI was calculated as weight (kg) divided by height (m^2).

Total expired ventilation ($\dot{V}E$)

VT, RR and $\dot{V}E$ were carried out using a Pneumotach airflow transducer (medium flow), BIOPAC Systems, USA.

Respiratory muscle strength

MICRORPM®, Medical, UK was used to measure inspiratory and expiratory muscle strength. All participants underwent maximal inspiratory pressure at residual volume (PImaxRV) and at function residual capacity (PImaxFRC), sniff nasal pressure (Pnsn) and maximal expiratory pressure (PEmax) evaluation. All of procedures are referenced base on American/European Respiratory Society "ATS/ERS Statement on Respiratory Muscle Testing"[25]. Each testing lasted at least 1.5s and was measured at least 5 times or more than that, until the closed highest 2 values were achieved. The highest value was recorded. RMS was calculated as [PImaxRV+PEmax]/2.

Statistical analysis

Data were expressed as means±SD and median. The Stata 10 Statistical software was used to perform the statistical analysis. Unpaired t-test was used to compare differences in characteristics and all parameters between obese and lean women. Two-sample Wilcoxon rank-sum (Mann-Whitney) test when data showed departure from normality was used. A value of $p<0.05$ was taken to be the threshold of statistical significance.

Results

Anthropometric data are summarized in Table 1. It was apparent that the obese women had almost identical mean age to normal-weight peer with the mean of approximately 32 yr. Compared with the lean individuals, obese women had significantly higher body weight ($p<0.001$), waist circumference ($p<0.001$), hip circumference ($p<0.001$) and WHR ($p=0.002$) compared with lean counterparts. BMI was greater in obese (BMI; 32.9±4.5, median 31.5 vs. 20.1±1.1, median 19.85 kg/m^2, $p<0.001$). In addition, the VT in obese women was higher (0.6±0.1 vs. 0.5±0.1 L, $p=0.006$) than that of the lean group. Nevertheless, RR and $\dot{V}E$ were not significantly different between the two groups.

Table 2 and Fig. 1 summarize data in regard to the RMS. It was observed that PImaxFRC, PImaxRV, Pnsn and PEmax in obese women were 22.9 cm H_2O ($p<0.001$), 34.6 H_2O ($p<0.001$), 13.9 H_2O ($p=0.01$) and 32.9 H_2O ($p<0.001$), respectively, greater than those of normal-weight women. Besides, obese women had stronger respiratory muscles indicated by a RMS of 33.7 H_2O higher than that of normal-weight women (Table 2 & Fig. 1).

Table 1 Characteristics of the study population

	Obese (n=16)		Normal-weight (n=16)		
	mean±SD	Median	mean±SD	Median	P
Age (years)	32.0±8.6	28.3	32.2±8.7	29.2	NS
Weight (kg)	79.8±11.3	80.2	49.7±3.42	49.8	<0.001
Height (m)	1.56±0.04	1.6	1.57±0.04	1.6	NS
BMI	32.9±4.5	31.5	20.1±1.1	19.9	<0.001
Waist (cm)	98.3±10.4	96.1	72.0±5.3	70.0	<0.001
Hip (cm)	112.1±7.1	113.5	90.5±4.9	91.5	<0.001
WHR	0.88±0.07	0.9	0.80±0.06	0.8	0.002
VT (L)	0.6±0.1	0.6	0.5±0.1	0.5	0.006
RR (/min)	16.0±4.2	15.0	17.0±3.6	16.0	NS
$\dot{V}E$ (L/min)	9.1±2.5	8.4	8.1±1.8	8.1	NS

BMI, body mass index; WHR, waist to hip ratio; VT, tidal volume; RR, respiratory rate; $\dot{V}E$, minute ventilation. Values are mean±SD and median tested by two-sample Wilcoxon rank-sum (Mann-Whitney) test. Age, weight, BMI, waist, hip, RR and $\dot{V}E$ tested by Mann-Whitney

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Table 2 Comparison of respiratory muscle strength between groups.

	Obese (n=16)		Normal-weight(n=16)		
	mean±SD	Median	mean±SD	Median	P
PImaxFRC	111.0±27.0	111.5	78.1±15.5	77.5	<0.001
PImaxRV	122.1±27.8	120.0	87.5±18.2	85.5	<0.001
Pnsn	90.2±11.8	92.0	76.3±20.3	74.0	0.01
PEmax	126.5±27.9	124.0	93.6±17.3	91.5	<0.001
RMS	124.3±21.2	124.0	90.6±17.1	88.0	<0.001

PImaxFRC, maximal inspiratory pressure from function residual capacity (cmH_2O); PImaxRV, maximal inspiratory pressure at residual volume (cmH_2O); Pnsn, sniff nasal pressure (cmH_2O); PEmax, maximal inspiratory pressure (cmH_2O) and RMS, respiratory muscle or [PImaxRV+PEmax]/2. Values are mean±SD and median tested by two-sample Wilcoxon rank-sum (Mann-Whitney) test. PImaxFRC and PImaxRV tested by Mann-Whitney.

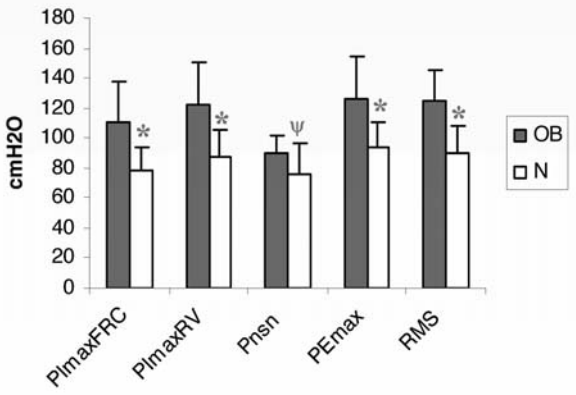

Figure 1. Respiratory muscle strength in 16 obese (OB) and 16 normal-weight (N) women. Abbreviations are as in Table 2. * p<0.001 and ˠ p=0.01. Values are mean±SD and median tested by two-sample Wilcoxon rank-sum (Mann-Whitney) test.

Discussion

The present study provides evidence that respiratory muscle strength, e.g. PImaxFRC, PImaxRV, Pnsn, PEmax and RMS in obese women was significantly higher compared to normal weight peers. This is in lines with previous studies which have shown that peripheral muscles strength is greater in obese individuals than in healthy peers.[13,,26,28]. Moreover, a recent study done in obese women has reported higher RMS.[19] They suggested that greater fat free muscle mass may pay compensation for the increased work of breathing forced by obesity and hence removing of impairing muscle strength. Moreover, obese individuals generally have supplementary muscle mass and more power than do normal-weight adults.[29,30] In fact, some studies suggests that greater qualities of type II muscle fibers, especially type IIb fibers, which are related to low endurance and have greater power to perform physical acivities, may be responsible for an adaptation of skeletal muscle on chronic overload compulsory by obesity.[19, 30,31] It may be possible that obese women in the present study could have adaptation of respiratory muscles and/or have more of fat free mass leading to stronger respiratory muscles. In addition, obese women studied breathed at higher VT than normal-weight. It is thus also probable that deep breathing improved respiratory muscle strength as observed in COPD patients.[10, 20, 21,22,23] Nonetheless, our results are not in agreement with those reporting no changes[9,10,11,12,13,19] or reduced respiratory muscle strength.[14,15,16,17,18] Impaired respiratory muscle function in obesity may be caused by increased elastic load which the respiratory muscles are required to overcome during inspiration.[16] An overstretched diaphragm would place this respiratory muscle at a mechanical disadvantage, leading to decreased inspiratory muscle strength and efficiency [32] Additionally, decreased skeletal muscle glycogen synthase activity in obese subjects may be associated with decreased isokinetic skeletal muscle endurance [32], although it is not known if this phenomenon actually occurs in respiratory muscles. It is possible that degrees of obesity, methods of measuring RMS and not having sex separated in different studies contribute to debatable findings. Our study had the age of an obese woman which was almost identical to a normal-weight peer of the same age. We are confident that obese women had greater amount of lean mass and therefore greater RMS. Nevertheless, it is essential that further studies involving more patients should be conducted, separating degrees of obesity.

Conclusions

Women with obesity did not have impaired RMS but instead had stronger respiratory muscles. This could probably due to, at least, deep breathing forced to the obese women. Several factors may also be exaggerated on respiratory muscle strength in obesity such as duration of obesity, physical activity and fat free mass. However, a further study on larger population is required to verify our results.

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Acknowledgements

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Conflict of Interest: We hereby declare none.

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Origanal Article

Effect of tetramethylpyrazine on ionic currents in human coronary artery endothelial cell (HCAEC)

Kitinat Rodthongdee, Wattana B. Watanapa, Katesirin Ruamyod

Abstract

Tetramethylpyrazine (TMP) was reported to cause vasodilation and reduce arterial pressure. In vascular smooth muscle cells (VSMCs), TMP induced hyperpolarization by affecting VSMC ionic channels. Additionally, TMP could elicit endothelium-dependent pulmonary artery relaxation. Therefore, we hypothesized that TMP may similarly affect ionic currents of human coronary artery endothelial cells (HCAECs). Cultured HCAECs were studied using whole-cell patch clamp technique. Cells were stimulated with 600-ms pulses from -40 mV holding potential to -100, -80, -60 … +80 mV. TMP at 3 and 30 μM significantly decreased current densities at +60 mV to 73.0 ± 10.6 % (n=7) and 78.7 ± 5.8 % (n=7), respectively. To test the TMP effects on small- and intermediate-conductance Ca^{2+}-sensitive K^+ channels (SK_{Ca} and IK_{Ca}), inward rectifier K^+ channels and non-selective cation channels, specific blockers were used (100 nM apamin, 10 μM clotrimazole, 100 μM Ba^{2+} and 10 μM La^{3+}, respectively) When most channels were blocked, leaving primarily SK_{Ca} or IK_{Ca} currents, 30 μM TMP decreased the current density to 61.0 ± 20.0 % (n=3) and 50.3 ± 5.1 % (n=3). TMP did not affect currents through other channels in similar experiments. These data suggested that TMP could diminish HCAEC currents, and this effect could be mediated by SK_{Ca} and/or IK_{Ca} inhibition. Further investigation will elucidate the signaling pathways involved.

Keywords: *tetramethylpyrazine, potassium channel, non-selective cation channel, human coronary artery endothelial cell, whole-cell patch clamp*

Tetramethylpyrazine (TMP), or ligustrazine, an active ingredient found in the rhizome of *Ligusticum chaunxiong Hort.,* is widely used in traditional medicine as prescription for cardiovascular disease such as angina pectoris and myocardial ischemia, as well as cerebral ischemic syndrome.[1-3] Indeed, TMP was shown to induce reductions of both portal venous and systemic arterial pressures in portal hypertensive rats, acting as a vasorelaxant.[4,5] Another report described a direct relaxing effect of TMP on vascular tissues. In addition, the protective effect of TMP in acute coronary syndrome patients has been reported.[6] Previous studies showed that TMP decreased tension of rat thoracic aorta, pulmonary artery and coronary artery.[7-10] The effects of TMP have mostly been investigated in vascular smooth muscle cells (VSMC), showing that this substance could cause VSMC hyperpolarization, L-type Ca^{2+} channel inhibition and K^+ channel activation, causing lowered Ca^{2+} influx.[9,11,12] Few studies investigated the direct effects of TMP on endothelial cells. One report showed that TMP could induce endothelium-dependent relaxation in rat pulmonary artery by stimulating endothelial nitric oxide production.[13]

Production and release of vasodilators including nitric oxide from endothelial cells are known to be modulated by ion channels in these cells.[14-17] Several exogenous vasorelaxing substances, e.g. ginsenosides and bilobalide,[18-20] have also been shown to affect endothelial ion channels. To our knowledge, no report has addressed TMP effects on endothelial ion channels. Since coronary artery endothelial cells are a major player in the control of coronary artery tone [21] and are involved in the pathogenesis of coronary artery disease,[22-23] and since TMP has been shown to relax coronary artery, our objective, therefore, was to investigate the effects of TMP on ionic currents in human coronary artery endothelial cells (HCAECs), as well as identifying the type(s) of ion channels affected.

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Materials and Methods

Cell culture preparation

Primary culture of human coronary artery endothelial cells (HCAECs), in the third passage, was obtained from Lonza Walkersville Inc., USA (Clonetics® endothelial cell system). Only the 4th to 8th passages were used in the experiments to minimize variations induced by cell culture environment. For electrophysiological study, cells were plated on coverslips for ease of transfer to the recording chamber.

Electrophysiological recording

Chemicals and Solutions

All chemicals were purchased from Sigma-Aldrich, (St. Louis, Missouri, USA), except where noted otherwise. 2, 3, 5, 6-Tetramethylpyrazine (TMP) (>98% purity, $HClO_4$ assay) was obtained from Merck Chemicals, Germany. Deionized water was used as a solvent for all chemicals. The composition of the internal solution was modified from Zünkler et al.[24] and Brzezinska et al.[25] and contained (in mM): 140 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, 10 EGTA

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and 2 ATP; pH 7.2, adjusted with KOH. The ATP-containing internal solution was kept on ice until use, but no ATP regenerating system was present. The addition of ATP in this solution was not only for preserving cellular energy and increasing recording time, but also for blocking the ATP-sensitive K^+ channel. The control (bath) solution was similar to a physiological solution and contained (in mM): 145 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES and 10 glucose, 320 mOsmol/kg; pH 7.3, adjusted with NaOH.

Clear TMP crystals were obtained from Merck Schuchardt OHG, Germany. 10 mM TMP stock solution was prepared by dissolving in deionized water and diluted by external solution to various concentrations, ranging from 1-100 μM.

To identify the HCAEC channels affected by TMP, known channel blockers were used: 100 nM apamin (Apa) for small-conductance calcium-activated (SK_{Ca}) channel[12] (Sigma-Aldrich); 10 μM clotrimazole (CTZ) for intermediate-conductance calcium-activated (IK_{Ca}) channel[26] (Merck KGaA), 100 μM $BaCl_2$ for inward rectifier potassium (K_{ir}) channel[27] (Sigma-Aldrich), and 10 μM $LaCl_3$ for non-selective cation (NSC) channel[28] (Sigma-Aldrich).

Procedures

All experiments were done at 25-28ºC. HCAECs selected for recording were isolated and round-shaped, with intact cell membrane. After immersion of a micropipette containing internal solution into the bath, the pipette offset was adjusted to cancel out the liquid junction potential and the pipette resistance was determined. The cell capacitance and series resistance were determined and cancelled after gaining whole-cell access, following the formation of a gigaohm seal between the micropipette tip and the cell membrane. The holding potential was -40 mV which approximated the HCAEC resting membrane potential previously reported.[24] Test pulses were applied from the -40 mV holding potential to a test pulse for 600 ms, then returning to the holding potential. The test pulses started from -100, -80, -60 … to +80 mV to construct a current-voltage (I-V) curve afterwards. The inter-pulse interval was 1 s.

For each cell, at least two-minute equilibration was allowed after gaining whole-cell access before a control current was recorded. Then the cell was exposed to a test concentration of TMP for at least five minutes. TMP concentrations tested were 1, 3, 10, 30, and 100 μM. Attempts were always made to record the wash-out currents, i.e., currents on return to the control bath solution after a test TMP solution. However, wash-out currents could only be obtained in a few cells due to loss of gigaseal. To minimize the carry-over effect, a coverslip that had been exposed to a TMP concentration for more than five minutes (i.e. in a previous experiment) would be discarded.

In one-blocker experiments, the control and wash-out currents were recorded in the presence of a channel blocker. Test currents were recorded after a five-minute exposure to 30 μM TMP and the continuous presence of the same blocker.

In three-blocker experiments, HCAEC currents exposed simultaneously to three blockers were compared in the presence *vs* absence of 30 μM TMP, to confirm the effect of TMP on a suspected target channel. The sets of three blockers were: i) 10 μM CTZ + 100 μM Ba^{2+} + 10 μM La^{3+} (mostly SK_{Ca} remaining); ii) 100 nM Apa + 100 μM Ba^{2+} + 10 μM La^{3+} (mostly IK_{Ca} remaining); iii) 100 nM Apa + 10 μM CTZ + 10 μM La^{3+} (mostly K_{ir} remaining); and iv) 100 nM Apa + 10 μM CTZ + 100 μM Ba^{2+} (mostly NSC remaining) (See also under the x-axis labels of figure 3 C for a simplified representation of this protocol)

Data analysis

Currents selected for analysis must pass the following criteria: i) the seal resistance must be larger than 1 gigaohm; ii) the maximum voltage error must not exceed 4 mV; and iii) the current magnitude at +60 mV must be at least 25 pA.

Data were initially analyzed using the pCLAMP10 software (Molecular Devices, USA). Current amplitude was taken as the difference between the steady-state current during a test pulse and the holding current. Currents at -100 and +60 mV were chosen to represent the inward and outward currents, respectively. Outward currents were not represented by those at +80 mV, the largest depolarization tested, because gigaseals could not be maintained at this depolarization in some cells, and therefore data at this potential were not consistently reliable. Current density was calculated by dividing maximum current amplitude at -100 and +60 mV with the whole-cell capacitance, and reported as pA/pF. GraphPad PRISM® 5 program (GraphPad Solfware, San Diego, CA, USA) was used to plot I-V curves and bar graphs, as well as performing all statistical analyses.

All data were tested for normality using Komogorov-Smirnov test. For normally distributed data, Student paired *t*-test or one-sample t-test (for data expressed in percentage of control) was used for paired data, and ANOVA with post hoc Bonferroni test was used for multiple comparisons. For non-normally distributed data, Wilcoxon signed-rank test and Kruskal-Wallis followed by Dunn's multiple comparison test were used for paired and multiple comparisons, respectively. All data were presented as mean ± standard error of mean (SEM), taking P value < 0.05 as statistically significant.

Results

Effect of TMP on HCAEC whole-cell currents

HCAECs were exposed to control solution and then TMP at a concentration of 1, 3, 10, 30, or 100 mM. Figure 1 A displays an example of current traces from a cell recorded in control compared to those in 30 mM TMP. Contrary to our expectation, 30 mM TMP actually inhibited HCAEC outward currents. In addition, the wash-out currents could be successfully recorded in this particular cell, as shown in the lowest panel of the figure, confirming the reversibility of the TMP block. The current density-voltage (I-V) curves obtained from this cell are plotted in figure 1 B. Average I-V curves from experiments with 3 and 30 μM TMP

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are displayed in figure 1 C and D. At +60 mV, the mean current densities in control and 3 mM TMP solutions were 10.64 ± 2.39 and 7.41 ± 1.72 pA/pF, respectively (n = 7; p = 0.0837, paired t-test), and those in control and 30 mM TMP external solutions were 3.26 ± 1.06 and 2.48 ± 0.85 pA/pF (n = 7; p = 0.0686, paired t-test). When the remaining currents during TMP exposure were expressed as percentage of control (Table 1), significant decrease in ionic currents could be demonstrated in the presence of 3 and 30 mM TMP. The percentage of 3 and 30 μM TMP-treated currents compared to controls were 73.0 ± 10.6 % (n = 7) and 78.7 ± 5.8 % (n = 7), respectively (P = 0.0448 and 0.0104, respectively; one-sample t-test). On the other hand, none of the TMP concentrations tested could significantly affect the inward currents at -100 mV (data not shown). Apparently, TMP at 3 and 30 mM could significantly reduce outward HCAEC currents by almost 30%. Significant differences with 10 and 100 mM TMP could not be demonstrated, possibly due to a rather high variability in the data.

Table 1. Percentage of outward currents (at +60 mV) compared to control at various TMP concentrations tested.

[TMP] (μM)	n	% Control	P value
1	6	102.0 ± 23.1	0.9351
3	7	73.0 ± 10. 6	0.0448*
10	8	118.5 ± 17.5	0.3266
30	7	78.7 ± 5.8	0.0104*
100	10	82.4 ± 9.2	0.0888

Data are expressed as mean ± SEM. Kolmogorov-Smirnov test showed that all data were normally distributed. One-sample t-test was employed to test if the % control (or percentage remaining) was significantly different from 100% (or control currents). * $P < 0.05$; one-sample t-test.

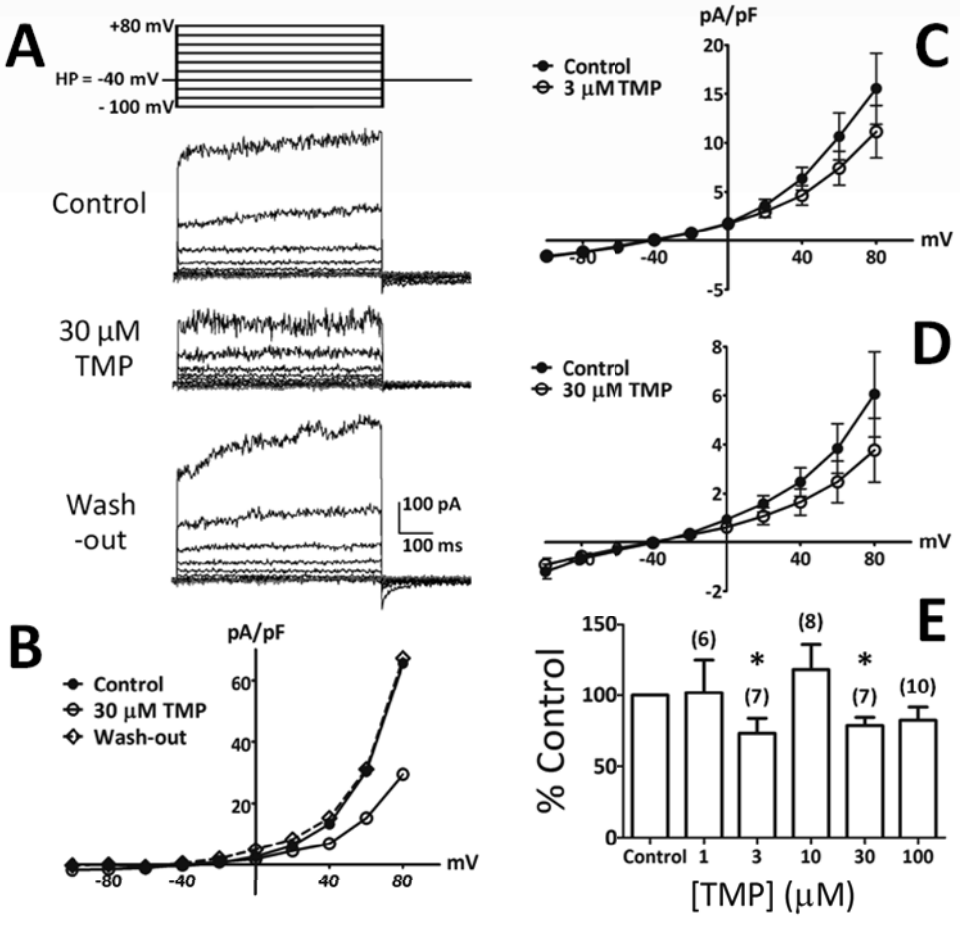

Figure 1. Tetramethylpyrazine (TMP) inhibits the outward currents of human coronary artery endothelial cells (HCAECs). A) An example of whole-cell currents obtained from an experiment with 30 μM TMP in an HCAEC with successful recording of wash-out currents. The uppermost panel diagrams the pulse pattern used in all experiments (*HP*, holding potential). *Control* and *Wash-out* currents were recorded in control external solution. **B)** current density-voltage (I-V) curves of the experiment in A, showing reversibility of TMP effect. **C)** and **D)** Average I-V relationships of control and 3 and 30 mM TMP-treated currents, respectively; error bars are SEM (n = 7 and 7). **E)** Bar graphs showing average TMP-exposed currents (at +60 mV), as expressed in percentages of control currents. The TMP concentrations were indicated on the x-axis. Error bars are SEM. The numbers in parentheses are sample size. Data are from Table 1. * $P < 0.05$ (one-sample t-test).

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One-blocker experiments

To identify the type of HCAEC ionic channels affected by TMP, experiments with 30 μM TMP were repeated in the continuous presence of either 100 nM Apa (SK_{Ca} blocker), 10 μM CTZ (IK_{Ca} blocker), 100 μM Ba^{2+} (K_{ir} blocker) or 10 μM La^{3+} (NSC blocker). The average outward currents (at +60 mV) remaining during exposure to 30 μM TMP, expressed as percentage of controls were 93.4 ± 12.6 % (n = 5, p = 0.6609), 109.7 ± 15.4 % (n = 7, p = 0.5540), 74.3 ± 13.8 % (n = 6, p = 0.1222), and 150.1 ± 46.3 % (n = 9, p = 0.7344), respectively (one-sample *t*-test) (figure 2). Therefore, no significant effects of TMP could be demonstrated in our one-blocker experiments.

Three-blocker experiments

Because the type of currents affected by TMP could not be concluded from the one-blocker experiments, a different approach was taken. Three blockers were present simultaneously in control external solution and the remaining HCAEC currents recorded, comparing control *vs* 30 μM TMP exposure. The blockers' concentrations were the same as above.

Average current density at +60 mV in the presence of CTZ, Ba^{2+} and La^{3+} (remaining currents were primarily through SK_{Ca}) in control and 30 μM TMP were 2.18 ± 0.69 and 1.32 ± 0.56 pA/pF, respectively (n = 3; I-V curves in figure 3 A), or the TMP-treated currents were 61.0 ± 20.0 % of control currents (figure 3 C). In Apa+Ba^{2+}+La^{3+} (remaining currents were mainly IK_{Ca}), control and 30 μM TMP-treated currents at +60 mV were 3.50 ± 0.25 and 1.78 ± 0.29 pA/pF, respectively (n = 3; I-V curves in figure 3 B), i.e., 30 μM TMP could decrease the outward currents to 50.3 ± 5.1 % (figure 3 C). When the remaining currents were mostly K_{ir} (in Apa+CTZ+La^{3+}), however, the control and 30 μM TMP- exposed currents were 13.73 ± 2.96 and 14.99 ± 3.52 pA/pF (n = 3), or currents in TMP were 108.6 ± 2.7 % of those in control (figure 3 C). Finally, in the presence of Apa+CTZ+Ba^{2+} (remaining currents were mostly NSC), the average current density in control and 30 μM TMP were 6.20 ± 0.97 and 6.53 ± 1.55 pA/pF (n = 4), and TMP-treated currents were 103.7 ± 16.3 % of control (figure 3 C). Although the sample size was too small for any reliable statistical analysis, due to the difficulty of finding cells with sufficiently large currents, these data hinted that 30 μM TMP may decrease SK_{Ca} and IK_{Ca} currents in HCAECs.

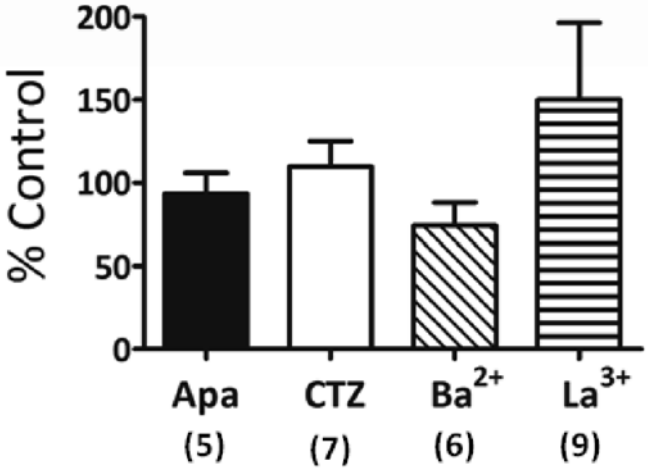

Figure 2. Bar graphs to summarize the results of one-blocker experiments. The x-axis indicates the blocker which was present continuously during recording of control, 30 μM TMP and wash-out currents. Y-axis represents the average percentage of remaining currents (at +60 mV) in 30 μM TMP compared to control currents. *Apa*, 100 nM apamin; *CTZ*, 10 μM clotrimazole; *Ba^{2+}*, 100 μM $BaCl_2$; *La^{3+}*, 10 μM $LaCl_3$. Error bars indicate SEM. The sample sizes are in parentheses.

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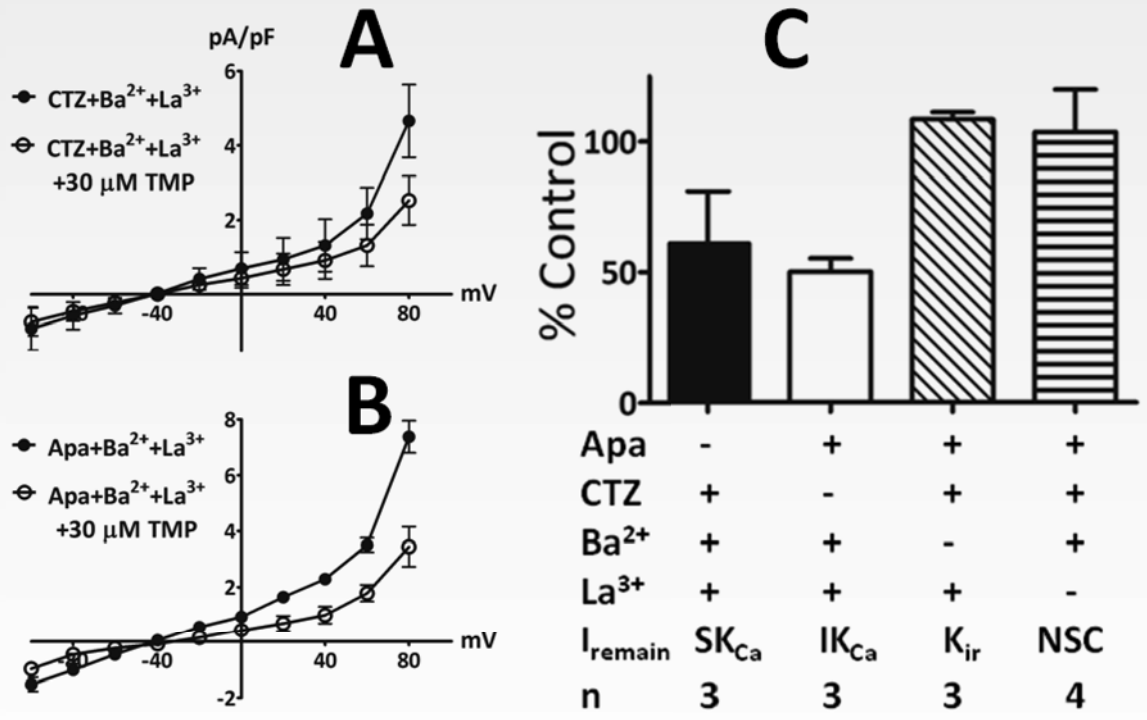

Figure 3. Three-blocker experiments. Average I-V curves of control and 30 μM TMP-exposed currents in the continuous presence of **A)** 10 μM clotrimazole + 100 μM Ba^{2+} + 10 μM La^{3+} (leaving mainly SK_{Ca} current; n = 3) and **B)** in control 100 nM apamin + 100 μM Ba^{2+} + 10 μM La^{3+} (leaving mainly IK_{Ca} current; n = 3). Error bars represent SEM; **C)** Bar graphs showing average 30 μM TMP-exposed currents (at +60 mV) as percentage of control currents when the indicated specific blockers were present; error bars indicate SEM. *Apa*, 100 nM apamin; *CTZ*, 10 μM clotrimazole; *Ba^{2+}*, 100 μM $BaCl_2$; *La^{3+}*, 10 μM $LaCl_3$; *+*, present; -, not present; *I_{remain}*, expected remaining current after three blockers had been applied; *n*, sample sizes.

Discussion

The present study showed that TMP could partially decrease HCAEC whole-cell outward currents at +60 mV, with significant inhibition demonstrated at concentrations of 3 and 30 μM. In one-blocker experiments, TMP could not inhibit outward currents in the presence of either 100 nM Apa (specific SK_{Ca} blocker[12]) or 10 μM CTZ (specific IK_{Ca} blocker[26]), while it may be able to do so, albeit without statistical significance, in the presence of K_{ir} blocker (figure 2). These results hinted at the involvement of SK_{Ca} and IK_{Ca} in mediating TMP's action. That statistical significance could not be reached may be due to the variability in our preparation.

Many studies suggested that endothelial currents were principally generated from NSC and K^+ channels, namely SK_{Ca}, IK_{Ca}, BK_{Ca}, K_{ATP}, and K_{ir}.[28] Since BK_{Ca}, or the large-conductance Ca^{2+}-activated K^+ channel, was shown to play minor role in endothelial cells,[29-31] it was not studied in our experiments. Addition of 2 mM ATP in all internal (pipette) solutions also excluded K_{ATP}. In our three-blocker experiments, inhibiting IK_{Ca}, K_{ir} and NSC channels, so that remaining currents were mainly SK_{Ca}, and subjecting the cells to 30 μM TMP, resulted in about 61 % average inhibition. Similarly, in the continuous inhibition of SK_{Ca}, K_{ir} and NSC channels, leaving primarily IK_{Ca}, channels, 30 μM TMP could decrease HCAEC currents, to approximately 50 % on average. However, when blockers were applied such that mostly K_{ir} or NSC channels remained, 30 μM TMP could not depress the average currents (average percentages of control in TMP were about 109 and 104 %, respectively). These data tended to be consistent with SK_{Ca} and IK_{Ca} being the channels inhibited by TMP.

Taken together, data from both one- and three-blocker experiments showed a trend which suggested that the specific types of ion channels involved may be SK_{Ca} and IK_{Ca}. This is in contrast to previous studies which suggested that 10 μM TMP could increase the SK_{Ca} current in vascular smooth muscle, causing smooth muscle cell relaxation.[9,12] The discrepancy may be due to different cell type (endothelial cells *vs* vascular smooth muscle), different type of vessels (coronary artery *vs* aortic smooth muscle; small *vs* large vessels), or different TMP doses (10 vs 30 μM TMP).

Outward currents in endothelial cells help keep the membrane potential negative, thus maintaining the driving force for Ca^{2+} influx through the endothelial cell membrane, resulting in increased vasodilator release.[14-17] Our results that TMP was inhibitory, rather than stimulatory, to endothelial outward currents means that the vasorelaxing effect of TMP, as shown in porcine coronary artery,[10] was the action of TMP mainly on vascular smooth muscle cells and the effect was not endothelium-dependent.

It remains to be tested whether higher doses of TMP could adversely cause vasoconstriction because of its action

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on endothelial cells. Further investigations are also needed to demonstrate the same effects in native (not cultured) human coronary artery endothelial cells. Finally, the involvement of BK_{Ca} and probably Cl^- currents, though minor in contribution, has not been ruled out by our experiments. This could be a cause of variability in our preparation and needs to be further studied.

Acknowledgements

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Conflict of Interest: None to declare

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Origanal Article

Identification of chalcone as an activator of CFTR chloride channel

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Abstract

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel expressed in epithelia of various organs, such as lung, pancreas and intestine. It is primarily located on the apical site of the cell membrane where it plays a key role in controlling fluid and electrolyte transport. Loss of functions of CFTR leads to cystic fibrosis (CF), a lethal genetic disease in Caucasians. The low activity of CFTR results in an excessively viscous mucous and impaired mucociliary clearance, making CF patients more vulnerable to recurrent infections with bacteria, which eventually end up with respiratory failure. Activators of CFTR therefore represent a promising therapeutic approach for cystic fibrosis. Recently, it was found that flavonoids, a plant-derived compound, stimulate CFTR activity in Calu-3 cells. In this study we aimed to investigate the effect of chalcone, intermediates of flavonoid biosynthesis, in modulating CFTR activity in a heterologous expression system of human CFTR, the Fisher rat thyroid cells stably transfected with human CFTR. Apical chloride current analysis showed that a representative chalcone, ASCM-007, stimulated CFTR-mediated chloride current primed by low dose of forskolin, an adenylate cyclase activator. Maximum response was obtained at 100 μM of ASCM-007. Due to the fact that the function of CFTR is regulated by intracellular cAMP levels, effects of ASCM-007 on intracellular cAMP levels were investigated by an immunological assay of cAMP. We found that ASCM-007 did not have an effect on intracellular cAMP levels. In addition, cell viability assay showed that ASCM-007 at concentrations of 10-100 μM had no cytotoxicity to FRT cells. In conclusion, the present study identified chalcone as a novel chemical class of CFTR activators.

Keywords: *CFTR, CFTR activator, natural products, cystic fibrosis, flavonoid*

Cystic fibrosis transmembrane conductance regulator protein (CFTR) is a cAMP-activated chloride channel expressed in epithelia of various organs including airways, intestine, kidney, pancreas, uterus and testes, where it mediates chloride transport. CFTR is classified in an ATP-binding cassette (ABC) transporter family. It is composed of 2 six-helix membrane spanning domains, each of which is followed by a nucleotide binding domain, NBD1 and NBD2 respectively. These NBDs contain sites for ATP binding and hydrolysis. Between the first NBD, NBD_1, and the second membrane spanning domain is a regulatory (R) domain containing multiple phosphorylation sites for protein kinases (1). In order to perform its function, CFTR must be phosphorylated on its R domain by protein kinase A (PKA), followed by the opening and closing of the channel induced by ATP binding and hydrolysis at both NBDs. As a stimulator of PKA, cAMP is therefore a key regulator of CFTR functions in the cells.

In respiratory tissues, CFTR is mostly found in serous submucosal glands where it mediates chloride-driven fluid secretion into the luminal surface of the airway maintaining optimal hydration and height of the airway surface liquid (ASL). Optimal height of ASL is required for normal airway mucociliary clearance of pathogens in the lungs. A CFTR mutation at phenylalanine residue 508 (ΔF508), which is located in NBD1 of CFTR, is the most common mutation found in cystic fibrosis (CF) patients (2). The mutant CFTR has abnormality in its folding and is therefore degraded by ER quality mechanisms, resulting in reduced CFTR expression and impaired chloride permeability of plasma membrane of airway cells. Low chloride secretion in CF causes excessively viscous mucous and impaired mucociliary clearance, making CF patients more vulnerable to recurrent infections with bacteria, especially *Psudomonas aeruginosa*. Activators of CFTR are therefore speculated to comprise promising therapeutic approaches for CF.

Recently, it was found that a plant-derived polyphenolic compounds such as flavonoids are potent activator of CFTR. Some of them, such as genistein and apigenin, were shown to activate CFTR via a mechanism involving their direct interaction with nucleotide binding domains (3-5). Chalcone, an intermediate of flavonoid biosynthesis, shares many biological activities to those of flavonoids. We therefore hypothesized that chalcone may have stimulatory effects on CFTR. In the present study, the effect of a representative chalcone, ASCM-007, isolated from flowers of *Butea monosperma* (Lam.) Taub. on CFTR activity was investigated in the Fisher rat thyroid (FRT) cells stably transfected with human CFTR.

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Materials and Methods

Cell line and Chemicals

Fisher rat thyroid (FRT) cells stably expressing human wild-type CFTR were generated as described previously (4). ASCM-007 was kindly provided by Prof. Apichart Suksamrarn (Ramkhamhaeng University, Bangkok, Thailand). Others chemicals were purchased from Sigma-Aldrich (Missouri, USA)

Cell culture

FRT cells were cultured in Coon's F-12 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown in a humidified incubator under conditions of 5% CO_2/95%O_2 at 37°C. For apical chloride current measurements, cells were subcultured in 0.05% trypsin EDTA solutions and then cultured on Snapwell inserts of 1 cm^2 surface area (Corning-Costar, Action, New York, USA) at the initial density of 500,000 cells/inserts. They were grown for 7 days to obtain an electrical resistance of greater than 1000 Ω/cm^2. Culture medium were freshly replaced daily.

Apical chloride current measurements

After obtaining desire electrical resistance, a Snapwell insert containing FRT cell monolayers was mounted between two hemichambers of Ussing chamber systems. In apical chloride current measurement, the basolateral hemichamber was filled with 5 ml of Ringer's solution containing (in mM) 130 NaCl, 2.7 KCl, 1.5 KH_2PO_4, 1 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES and 10 glucose (pH 7.4). In the solution bathing the apical hemichamber, 65 mM NaCl was replaced by 65 mM Na-gluconate, and the $CaCl_2$ concentration was increased to 2 mM. Solutions in both hemichamber were bubbled with oxygen and temperature was maintained at 37°C throughout the experiment by temperature-controlled circulating water bath. The basolateral membrane was permeabilized by amphotericin B (250 μg/ml) for 30 min before the apical chloride current measurement. Apical chloride current measurements were recorded using a DVC-1000 voltage-clamp (World precision Instruments, Sarasota, FL) with Ag/AgCl electrodes and 1 M KCl agar bridges.

Intracellular cAMP measurements

Intracellular cAMP contents were measured using an R&D System's cAMP immunoassay (R&D Systems, Minneapolis, USA). Briefly, FRT cells (1×10^6 cells/well) were cultured overnight in 24-well plates at 37°C under conditions of 5%CO_2/95%O_2. After removal of culture medium, cells were washed with PBS for 2 times, incubated with 0.1% DMSO (control) or 100 μM of ASCM-007 for 30 minutes. After 30 minutes of treatment, cells were lysed with cell lysis buffer 5 provided by manufacturers, freezed at ≤ –20°C, and thawed with gentle mixing. The freeze/thaw cycle was repeated for 2 times. Lysates were transferred into primary antibody coated 96-well plates. The assay was performed in duplicate in at least three separate experiments and titrated so that the data will be within linear ranges of standard curves according to manufacturer's instructions. A yellow color developed by addition of 100 μl of 2 N sulfuric acid was measured by determination of an absorbance at 450 nm within 30 minutes using a spectrophotometer (EL 312, Bio-Kinetics Reader; Bio-Tek Instruments Inc., Helsinki, Finland).

Cell viability assays

Effects of ASCM-007 on cell viability were determined using an MTT assay. Briefly, FRT cells were counted by hemocytometer and evenly distributed into 96-well plates at a density of 5×10^5 cells/well. After growing the cells for 24 hours in a humidified 5 % CO_2 incubator, ASCM-007 at various concentrations were added into the culture medium with the final volume of 200 μl per well. 24 hour afterwards, the cells were stained for 4 hours at 37°C with 20 μl of MTT reagents. Then, 150 μl of DMSO was added to stop the reaction before the measurement of an absorbance at 590 nm by a spectrophotometer (EL 312, Bio-Kinetics Reader; Bio-Tek Instruments Inc., Helsinki, Finland).

Statistical analysis

Results were presented as mean ± SE Statistical difference between control and treatment groups was determined using Student's t test, with P value < 0.05 being considered to be statistically significant.

Results

Activation of CFTR activity by ASCM-007

The effect of ASCM-007 on CFTR activity was examined by measuring the apical chloride current in FRT cells stably expressing human wild-type CFTR. In this experiment, basolateral membrane of FRT cells was permeabilized by amphotericin B in the presence of apically directed chloride gradient to allow a direct measurement of chloride transport through CFTR principally located in the apical membrane of these cells. Forskolin at low concentration (1 μM) was added into both sides of hemichambers in order to provide minimal CFTR phosphorylation required for subsequent CFTR stimulation. After stabilization of the forskolin-stimulated chloride current, ASCM-007 at concentrations ranging from 10-100 μM was added into both sides of the hemichambers. As shown in figure 1, ASCM-007 stimulated CFTR-mediated apical chloride current in a dose-dependent manner. The maximum response was obtained at 100 μM of ASCM-007. We also tested the effect of ASCM-007 at higher concentrations, but there was no further stimulatory effect observed (data not shown).

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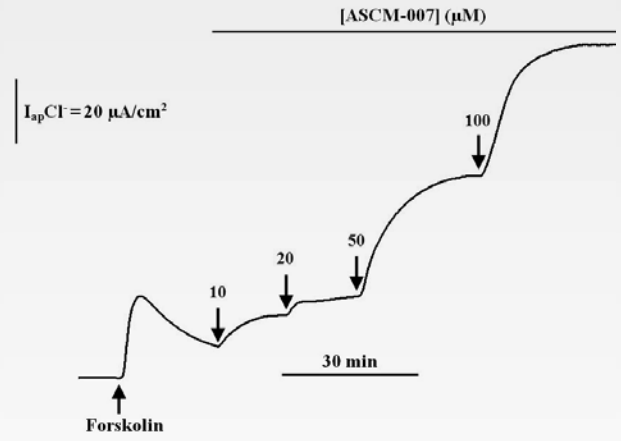

Figure 1. Effect of a representative chalcone, ASCM-007, on CFTR functions. (A) After stimulation of CFTR-mediated chloride current with low concentration of forskolin (1 μM), accumulative doses of ASCM-007 were added into both apical and basolateral solutions. A representative current tracing of 5 separate experiments was shown. (B) A summary of dose-response relationship of stimulatory effect of ASCM-007 on apical chloride current in FRT cells. Data was expressed as mean of % activation ± SE, *n* = 5

No involvement of changes in intracellular cAMP contents in ASCM-007's action

An increase in intracellular cAMP could result in CFTR activation. To examine whether the CFTR stimulatory effect of ASCM-007 involves an alteration in intracellular cAMP levels, cAMP immunoassays were performed. FRT cells were treated with a vehicle (DMSO) or 100 μM of ASCM-007 and incubated for 30 minutes before measuring intracellular cAMP contents. It was found that ASCM-007 did not have any effects on intracellular cAMP levels compared to controls, as shown in figure 2. As a positive control, forskolin, an adenylate cyclase activator, stimulated intracellular cAMP levels by 10 folds (data no shown).

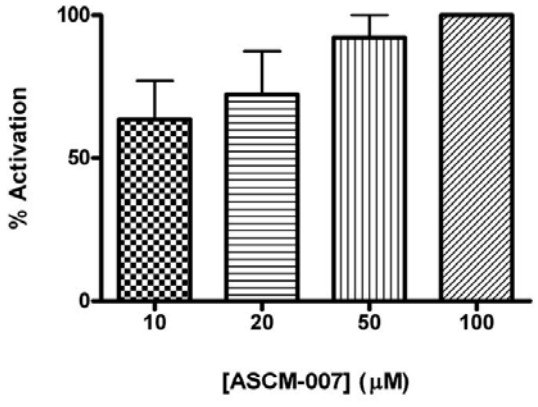

Figure 2. Effect of ASCM-007 on intracellular cAMP levels in FRT cells. Intracellular cAMP content after treatment with DMSO and ASCM-007 were measured by cAMP immunoassays. Data was shown as mean ± SE; *n* = 3. NS represents no statistical significance compared to control.

Effect of ASCM-007 on cell viability measured by MTT assays

Cytotoxicity of ASCM-007 in FRT cells was determined by MTT assays. FRT cells were incubated with ASCM-007 at various concentrations for 24 hours prior to MTT assays. In this assay, MTT is reduced by reductase enzymes present in living cells giving rise to a purple color of formazan. The numbers of live cells are quantified from the measurement of the absorbance by a spectrophotometer. The results showed that ASCM-007 at concentrations of 8-125 μM did not have any effects on the viability of FRT cells as shown in figure 3.

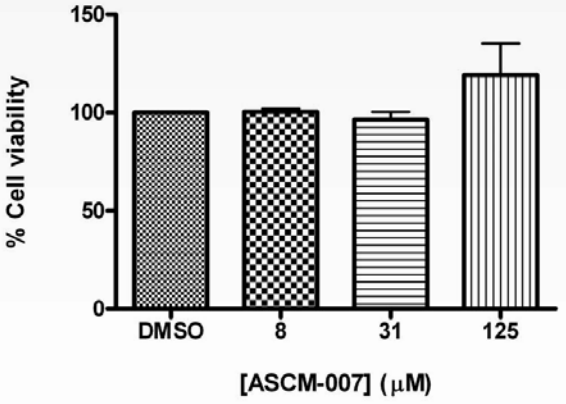

Figure 3. Determination of ASCM-007 cytotoxicity in FRT cells by MTT assays. FRT cells were incubated with ASCM-007 at indicated concentrations. Cell viability was assessed by determination of absorbance at 590 nm. Data was presented as mean of % control ± SE, *n* = 3. There is no significance difference between treatment and control groups (no ASCM-007).

Discussion

CFTR is a cAMP-activated chloride channel located in apical membrane of epithelial cells in many tissues. It belongs to an ATP binding cassette superfamily (6). CFTR is composed of 2 membrane-spanning domains (MSD), 2 nucleotide-binding domains (NBD) and a regulatory (R) domain (1). Activation of CFTR requires phosphorylation of the R domain by a cAMP-activated protein kinase A (PKA) (7). Besides CFTR phosphorylation, binding and hydrolysis of ATP at NBDs are required for opening and closing of CFTR. The loss of CFTR function causes a lethal disease cystic fibrosis, which is a genetic disorder commonly found in Caucasians. Cystic fibrosis occurs by a mutation at ΔF508 residues in one or both of CFTR alleles (6). This mutation affects to the function and expression of CFTR and leads to improper mucociliary clearance in the lung, resulting in bacterial infection and respiratory failure. Nowadays, CFTR activator is considered to be a potential way for cystic fibrosis treatment. To date, a variety of natural products, including flavonoids, have been identified as CFTR activators.

Flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. They were reported to have many biological effects such as antibacterial, anti-inflammatory and antiviral activities

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(8, 9). In addition, in 1995 Illek and coworkers found that genistein, a flavonoid derivative found in soy-rich diet, activated CFTR functions without affecting phosphodiesterase and PKA activities (3). In the present study, we hypothesized that chalcone; an intermediate of flavonoids biosynthesis may have an effect on CFTR function.

In this study, ASCM-007, a representative of chalcone, was used to investigate its stimulatory effect on CFTR. Apical chloride current was measured in Fisher rat thyroid (FRT) cells stably expressing human CFTR as these cells have negligible expression of non-CFTR chloride channels. In addition, these cells form epithelial monolayer on Snapwell insert with electrical resistance high enough to perform electrophysiological studies (4). The results showed that ASCM-007 activated CFTR function in dose-response manner. Even though, it was shown previously that no other chloride channels except CFTR was detected electrophysiologically in the FRT cells over-expressing human CFTR, further studies are required to confirm effects of this compound on CFTR using specific CFTR inhibitor or non-transfected FRT cells. In addition, due to the fact that losing of tight junction integrity during the experiment by cell death might affect the apical chloride current measurements, we therefore investigated cytotoxic effect of ASCM-007 on FRT cells. Our data showed that ASCM-007 at the concentrations we used in the apical chloride current measurement did not have cytotoxic effects on FRT cells. There are several possible mechanisms accountable for CFTR stimulatory activities of this compound, including the elevation of intracellular cAMP. In general, intracellular cAMP levels are controlled by the activities of adenylate cyclases and nucleotide phosphodiesterases, which are responsible for the production and degradation of cAMP respectively. In order to examine whether ASCM-007 exerted its effect via inducing a change in intracellular cAMP, we measured cAMP contents and found that ASCM-007 did not change the intracellular cAMP content. This result suggests that the target of ASCM-007 should be downstream of cAMP production. In conclusion, this study identified a novel class of the activator of CFTR chloride channels. Further development of this class of compounds may provide a new therapeutic intervention for cystic fibrosis and other disease involving CFTR hypofunction.

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Conflict of Interest: There is no conflict of interest in this study.

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O-01

Biodegradable nanocomposites for tissue adhesive

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Nanocomposites possess several advantages for tissue adhesive: high water resistance that can be uses in the human body, direct bonding immediately on the glass surface in water for long time, high bonding strength to living tissue, biocompatibility and bioabsorbable, low cost, eco-friendly green nanocomposites. The main objective of this work was to make tissue adhesive agents from rice starch, gelatin and nanopowder composites then to test the final products for their scientific and medical properties as per the Thai Ministry of Health, in order to demonstrate that the product(s) can be safely used. The principal raw materials were pharmaceutical grade Thai rice starch powder and analytical grade gelatin powder. The additives were hydroxyapatite (HA) nanopowder or carbon nanopowder, carboxy- methylcellulose and lactic acid. All materials were milled in a ball mill pot for 24 h then solubilized in water at 60°C and stirred until homogeneous. The suspension was put polystyrene culture dishes and dried at 60°C for 48 h. The binding characteristics of the final products were then investigated by scanning electron microscopy (SEM). The SEM images clearly showed the nanocomposite materials. The best tissue adhesive agent comprised rice starch 3 g, gelatin 1.5 g, sodium carboxymethylcellulose 0.2 g, lactic acid 5 mL and carbon nanopowder 0.1 g. Its (a) average swelling ratio (%) was 2.44±0.34% (b) average time taken to bond on a glass surface in water 109.2±10.6 minute and (c) average bonding strength to tissue of porcine 0.210±0.005 MPa.

Keywords: *bioadhesives, hydrogels, nanocomposites, biodegradable*

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O-02

Mechanical properties of sintered submicrometer hydroxyapatite-bioglass composite

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The mechanical properties of hydroxyapatite ceramics were improved by adding 5 wt% of Ca-P-Na-based-glass (powdered to submicron size) as a sintering aid. Microstructure and mechanical properties of the composite products were subsequently investigated. The hydroxyapatite composites had a bulk density of 3.02±0.01 g/cm^3, a porosity of 0.01±0.002%, a microhardness of 425±11.6 HV and a flexural bending strength of 109.0±2.7 MPa. This bending strength represented an increase of about 42% in the sample fabricated by micron-sized HA composite powder having the same porosity. These values are in range of the strength of cortical human bone. Thus, a submicron-scaled HA composite can be produced with improved mechanical properties for use as a high-load-bearing bone substitute. The Young's modulus values (16.4±1.8 GPa) matched well with the Young's modulus values for human bone, so it should not cause interfacial stress between the implant and bone, resulting in good fixation.

Keywords: *bioceramics, bone implants, bovine bone, microstructure, hydroxyapatite*

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O-03

Effects of banana (*Musa sapientum* L.) supplemented diet on spatial learning and memory in male rats

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For many years banana (*Musa sapientum* L.) has played an important role in Thai infant nutrition. Thai traditional folklore suggests that feeding bananas to infants can enhance their memory and learning capability. The present study aimed to examine the effect of a banana supplemented diet on (a) spatial learning and memory and (b) hippocampal neurogenesis using rats as an animalmodel. Weaned male Sprague-Dawley rats (postnatal day 19-21) were fed with either the control (CON) diet or the 3% (SUP1) or 6% (SUP2) banana supplemented rat diet. The results indicated no significant difference in body mass among the three weekly food intake groups. After 10 weeks, the rats were given 3 days of training (3 trials/day) in a Morris water maze and retention performance was examined 48 hours later. Time to reach the platform (latency in seconds) and total length of swimming path (distance in cm) were used as indices of spatial memory. Overall, there were no significant effects of diet (Kruskal-Wallis ANOVA on ranks), but SUP2 rats trended to have a shorter latency and both SUP1 and SUP2 rats tended to travel a shorter distance. Following the behavioral assessment, rats will be injected with Bromodeoxyuridine (BrdU; 50 mg/kg) for 5 days to label dividing cells. The rats will then be sacrificed and the numbers of newly proliferated nerve cells in the hippocampus of their brains estimated by immunohistochemical techniques. Potential roles of banana supplemented diet on the rats' spatial learning and memory and neurogenesis in the hippocampus will be presented and discussed.

***Keywords**: bromodeoxyuridine, hippocampus, Morris water maze, spatial memory*

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O-04

Mulberry fruit improves memory impairment and enhances cholinergic function in vascular dementia

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Vascular dementia is the second most common form of dementia after Alzheimer disease (AD); however, this condition can be prevented and is treatable. Recent findings showed that the polyphenolic compounds can protect against vascular dementia. We hypothesized that the extract of mulberry (or *Morusalba*) fruit, family Moraceae, might mitigate the memory impairment in vascular dementia. The current study was undertaken to determine the effect of alcoholic extract of mulberry fruit on memory and cholinergic function in the hippocampus of an animal model of vascular dementia. Male Wistar rats, weighing between 300 and 350g, were given mulberry fruit extract orally at various doses (2, 10 and 50 mg/kg BW) for 7 days before and 21 days after the occlusion of the right middle cerebral artery (Rt.MCAO). The cognitive enhancing effect of the animals was determined using the Morris water maze test every 7 days after the operation throughout the 21-day experimental period. The animals were then sacrificed to determine the density of cholinergic neurons and the activity of acetylcholinesterase (AChE), a key enzyme indicating the acetylcholine (ACh) turnover rate, in hippocampus. The medium and high doses of mulberry fruits extract decreased both escape latency and AChE activity. Only rats subjected to the high dose treatment showed any increased cholinergic neuron density in the CA3 of the hippocampus. Our data suggest that the cognitive enhancing effect of mulberry fruit extract might occur partly via an enhancement of cholinergic function. Mulberry fruit extract is a potential food to protect against vascular dementia. Further research is necessary before moving to clinical trials.

Keywords: *Mulberry fruits, Morusalba, vascular dementia, memory, cholinergic function*

Acknowledgements: This study was supported by The Queen Sirikit Department of Sericulture and Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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O-05

Wound healing activity of *Anacardium occidentale* in streptozotocin-induced diabetic rats

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The extract of leaves of*Anacardium occidentale* L. has been used in folk medicine to treat ulcers and burns. Recent studies showed that the extract protected against the diabetic condition and enhanced the wound healing process. We hypothesized that the alcoholic extract of *A. occidentale* leaves would enhance the wound healing process in diabetics, a considerable public health challenge. Thus, this study aimed to assess its wound healing effect on streptozotocin-induced diabetic rats, which had an induced excision wound 1 cm (width) ×1 cm (long) at the anterior-dorsal side at the T1 Level. *A. occidentale* leaves extract was administered topically at doses of 25, 100 and 200 mg/kg once daily throughout the 15-day experimental period. All of the rats were evaluated every 3 daysfor wound healing using (a) a wound score (b) a wound healing index and (c) histopathological changes as indices. The results showed that the extract at doses of 25 and 100 mg/kg BW significantly enhanced wound healing in diabetic rats. The optimum effect was observed at a dose of 25 mg/kg BW. This is the first study to demonstrate that *A.occidentale* is effectively in promoting diabetic wound healing, promising to serve as a health product for diabetics; however, further clinical trials are essential.

Keywords: *Anacardium occidentale leaves, diabetic wound*

Acknowledgements: This study was supported by the National Research Council of Thailand and the Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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O-06

Liver x receptors regulate human organic anion transporter 1 in renal proximal tubular cells

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Liver x receptors (LXRs) play an important role in the regulation of cholesterol and glucose homeostasis. LXRs regulate the function of several transporters such as ATP-binding cassette transporters and organic anion transporter 2 (OAT2). Accordingly, it is possible that the LXRs may regulate OAT1-mediated organic anion transport. In the current study, we investigated the role of LXRs on human OAT1 (hOAT1)-mediated organic anion transport. After exposing renal S2 cells expressing hOAT1 to LXR agonists (TO901317 and GW3965), their transport activities were significantly decreased in a dose- and time-dependent manner. The inhibitory effect of TO901317 on hOAT1-mediated ^{14}C-PAH transport was restored by co-incubation with 22(S)-hydroxycholesterol, a LXRs antagonist, indicating that the inhibitory effect of TO901317 worked via LXR activation. Additionally, kinetic analysis revealed that the reduction of hOAT1 activity following LXRs activation was mediated by a decrease in the maximum rate of PAH transport (J_{max}), which represents numbers of plasma membranes hOAT1. This result correlated well with data from Western blot analysis, which showed decreased hOAT1 expression following LXRs activation. Our findings indicate that hOAT1 was down-regulated by LXRs activation in renal proximal tubular cells. These data clarify the physiological role of LXRs in the organic anion profile of the body.

Keywords: *Liver x receptor, organic anion, transporter, kidney, para-aminohippuric acid*

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O-07

Neuroprotective and cognitive enhancing effects of *Anacardium occidentale* leaf extract

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Oxidative stress is theorized to play a crucial role in neurodegeneration and memory impairment. Thus, the neuroprotective and cognitive enhancing effects of substances possessing antioxidant activity have gained much attention. This study was conducted to determine the effect of alcoholic extract of *Anacardium occidental* L. leaves on neurodegeneration and memory impairment induced by AF64A, a cholinotoxin. Male Wistar rats, weighing between 200 and 250 g, were administered *A. occidentale* extract orally, at doses of 25, 100 and 200 mg/kg BW once daily for 7 days before and after the induction of memory impairment by the bilateral intracerebro ventricular administration of AF64A. The rats'spatial memory and non-spatial memory were tested, using Morris water maze test and the object recognition test, respectively. The memory assessment was performed after substance administration every 7 days and the cholinergic neurons density (in various hippocampal regions) was determined at the end of experiment. The results showed that the extract at doses of 25 and 100 mg/kg BW significantly mitigated the elevation of escape latency and the decreased retention time induced by AF64A. Moreover, all doses of the extract used in this study enhanced total exploration time at 6 h after administration while a significant effect at 30 minutes was observed only in the rats subjected to a medium dose treatment. The extract at a medium dose enhanced cholinergic neuron density in all areas of the hippocampus except in the dentate gyrus, while a significant change caused by ahigh dose was observed only in CA3. Our data confirm that *A. occidentale*can increase both cholinergic neuron density in the hippocampus and improve memory. *A.occidentale* may be beneficial for the elderly and memory impaired patients, but further researchis necessary.

Keywords*: Anacardium occidentale, spatial memory, non-spatial memory, AF64A*

Acknowledgements: This study was supported by the National Research Council of Thailand and Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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O-08

Chitooligosaccharide ameliorates inflammatory bowel disease through inhibition of NF-kB activation and oxidative stress-induced epithelial cell damage

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Chitooligosaccharide (COS) is an enzymatically degraded product of chitin, a biomaterial from crustaceans. COS has received considerable interest for biomedical applications due to their biocompatibility and nontoxic nature. Although a variety of biological activities of COS have been reported, its anti-inflammatory activity remains unexplored. The current study aimed to investigate the anti-inflammatory effects and potential applications of COS in the treatment of inflammatory bowel disease (IBD), using both *in vivo* and *in vitro* models. Mouse models of experimental colitis induced by ingestion of 5% dextran sulphate sodium and intrarectal infusion of 4% acetic acid were used to evaluate the therapeutic efficacy of COS. Compared to colitis control groups, mice treated with COS (20 and 100 mg/kg/day) had (a) an increased survival rate (b) a decreased disease activity index (DAI) score and (c) a decreased histological index (HI) score. Notwithstanding, a higher degree of improvement was observed in the group receiving a low-dose of COS. Similarly, basal transepithelial resistance was preserved in colitis mice treated with COS compared with the controls. Furthermore, COS markedly decreased the activity of myeloperoxidase (MPO), an index of neutrophil infiltration, and inhibited activation of NF-κB in colonic tissues of colitis mice. Pretreatment of human intestinal epithelial cells (T84 cells) with COS attenuated both H_2O_2 and TNF-α-induced cell damage, in a dose-dependent manner without affecting cell proliferation. These results suggest that COS protects against IBD through inhibition of NF-κB signaling and protection against oxidative stress. This study provides novel insight into potential applications of COS for treatment of IBD and other inflammatory disorders.

Keywords: *chitooligosaccharides, inflammatory bowel disease, NF-κB, anti-inflammatory*

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O-09

Liver X receptors agonists attenuate arginine vasopressin-induced chloride secretion in MDCK-C7 cells

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP binding cassette (ABC) transporter and is reportedly responsible for chloride secretion in polycystic kidney disease (PKD). Liver X receptors (LXRs) are members of a group of nuclear receptors (NRs) that play important roles in lipid and carbohydrate metabolism. The activation of LXRs increases an ABC transporter, ABCA1, gene expression level that functions as a cholesterol pump. It is possible, therefore, that LXRs may regulate CFTR mediated-chloride secretion in renal tubular cyst development and enlargement in PKD. To evaluate this effect, polarized MDCK-C7 cells were treated with LXRs agonists at various concentrations and time intervals followed by short circuit current (I_{sc}) measurement that refers arginine vasopressin (AVP)-induced chloride secretion. Exposure of MDCK-C7 cells with 5 mM of TO 901317 or 2μM GW3965 for 24 hours led to reduction of chloride current to ~45.27±4.84% and 63.43±12.59% of control, respectively. The acute effect of LXRs on AVP-induced chloride secretion could not, however, be seen. These results suggest that the effect of LXRs on chloride secretion was mediated by genomic action. Basolateral-permeabilized MDCK-C7 cells showed that the apical membrane was a target of the inhibitory effect of LXRs on chloride secretion. These results correlated well with evidence that LXRs down-regulated CFTR protein expression, as evaluated by western blot analysis. This is the first evidence to show that LXRs play a role in the regulation of CFTR in renal tubular cells.

Keywords: *liver X receptors, chloride secretion, arginine vasopressin, MDCK-C7 cells, CFTR*

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O-10

Tetrahydrocurcumin protects against cadmium-induced endothelial dysfunction in mice

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Cadmium (Cd) is a serious industrial and environmental pollutant. Its toxicity and adverse effects have evoked concern regarding the consequences of Cd exposure to animals and humans. Recent advances in cadmium toxicity research suggest an association between cadmium and cardiovascular diseases (CVD); the mechanisms and implications of which remain unexplained. Tetrahydrocurcumin (THU) is a major metabolite of curcumin and has a variety of biological activities as curcumin. Although THU possesses a strong antioxidant effect, its potential value in CVD, especially in toxic metal-induced hypertension has not been evaluated. The current study aimed to determine the ability of THU to protect against Cd-induced endothelial dysfunction in male ICR mice. Mice received cadmium chloride (100 mg/L) via their drinking water for 8 weeks and THU was intragastrically administered at doses of 50 and 100 mg/kg/day with concurrent Cd treatment. The results showed that Cd increased blood pressure and attenuated vascular responses to acetylcholine, phenylephrine and sodium nitroprusside. Cd suppressed endothelial nitric oxide synthase (eNOS) protein expression in aortic tissue, which further suggests that Cd impairs endothelial function. Interestingly, THU supplementation significantly reduced blood pressure and restored vascular responsiveness and increased eNOS protein expression in a dose-dependent manner. Thepresent study provides evidence of a protective role for THU in ameliorating Cd-induced endothelial dysfunction in mice.

Keywords: *tethrahydrocurcumin, cadmium, endothelial dysfunction, nitric oxide*

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O-11

Passive smoking and risk of cervical cancer in northeast Thai women

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Tobacco smoking increases the risk for many types of cancer. The relationship between passive smoking and cervical cancer susceptibility among Northeast Thai women was determined in this study**.** Subjects included 138 cases with squamous cell carcinoma of the cervix (SCCA) and 138 age-matched healthy controls. Cervical HPV DNA was analyzed with the GP5+/6+ primer. Information including sexual behaviors, reproductive history, contraceptive use and history of smoking was obtained from a self-reporting questionnaire as well as a direct interview. The prevalence of HPV infection in the control and SCCA patients was 13.45% and 88.68%, respectively. Age at the first delivery, smoking status of sexual partners, prolonged oral contraceptive pills use, age at the first sexual intercourse, numbers of pregnancies and sexual partners revealed a higher risk for cervical cancer with a respective odds-ratio of 4.06, 3.58, 3.35, 2.79, 2.08 and 2.00, respectively ($p < 0.05$). In addition, the smoking habit of partners was associated with carcinogenesis in cervical cancer patients. Long-term exposure to a smoking partner (> 20 years), smoking > 20 cigarettes per day, and exposure time to passive smoking (≥ 5 hours per day) increased the risk of cervical cancer 3.09-, 2.36- and 5.22-fold, respectively ($p < 0.01$). Our data suggest that HPV infection, oral contraceptive pill use, and age at the first delivery were associated with cervical carcinogenesis among Northeast Thai women. Our findings also demonstrate that cervical carcinogenesis can be attributed to passive smoking.

Keywords: *cervical cancer, passive smoking, risk factor, HPV*

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O-12

Effect of ginsenoside Re on whole-cell currents of human coronary artery endothelial cells

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Endothelial cells are important in regulating coronary circulation, by secreting vasodilators and/or vasoconstrictors. Ginsenoside Re (Re), an active component in ginseng, was reported to increase NO secretion from human umbilical vein endothelial cells. In other cell types, this compound was found to increase K^+ current or Ca^{2+}-sensitive K^+ (K_{Ca}) current, leading to nitric oxide synthase (NOS) stimulation, increased NO secretion and vasodilation. Re, therefore, may increase K_{Ca} activity in human coronary artery endothelial cells (HCAECs). HCAECs exposed to different concentrations of Re were studied using the whole-cell patch clamp technique. The effect of Re on K_{Ca}, non-selective cation (NSC) and inward-rectifier potassium (K_{ir}) channels were investigated by using specific blockers. All currents were reported as mean ± SEM. Re dose-dependently increased outward currents (EC50=371.5±2.92 nM; $p<0.05$). 1μM Re could significantly increase outward currents by 28.93±7.54% when NSC channel was blocked by La^{3+}, but failed when the small-conductance K_{Ca} (SK_{Ca}) channel was inhibited by apamin. When the respective NSC, inward rectifier, intermediate- and high-conductance K_{Ca} channels were simultaneously blocked (with La^{3+}, Ba^{2+}, clotrimazole and TEA), Re was still able to increase outward currents significantly (35.49±4.22%); this effect was again abolished by apamin. These results indicate that Re increased HCAEC outward currents by opening SK_{Ca} channels, therefore, ginsenoside Re may also cause coronary vasodilation in humans, adding to the benefits of ginseng, with the promise of protection and/or treatment against coronary artery disease and other cardiovascular conditions.

Keywords: *Ginsenoside Re, calcium-activated potassium channels, human coronary artery endothelial cells*

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O-13

Screening of total phenolic compound, anti-oxidative properties and neuropharmacological activities of *Moringa oleifera* Lam. leaf extract

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Moringa oleifera Lam. (MO), family Moringaceae, has long been used as a food and traditional medicine in Thailand. *M. oleifera* is claimed useful for the treatment of numerous ailments including asthma, gout, lumbago, rheumatism, enlarged spleen and liver. Nevertheless, few studies have evaluated its polyphenolic compound, anti-oxidant and neuropharmacological activities. The purpose of this study was to evaluate the total phenolic compounds, anti-oxidative properties and neuropharmacological activities; including anxiolytic, antidepressant and cognitive enhancing activities of the alcoholic extract of *M. oleifera*. The total phenolic compound was determined using the Folin-Ciocalteu reagent; the anti-oxidant activity using the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the ferric reducing/antioxidant potential (FRAP); and the total phenolic compound, DPPH radical scavenging activity and FRAP activity by the 340 mg/L Gallic acid equivalent, 96.49±0.87 μg/mL and 857.70±6.07 μM L-ascorbic acid equivalent, respectively. The extract was further investigated for the anxiolytic, anti-depressant and cognitive enhancing affects using elevated plus maze, forces swimming and Morris water maze test, respectively. For the latter, male Wistar rats (250-300 g) were given the leaf extract orally at doses of 100, 200 and 400 mg/kg for 14 days. Then, the activities mentioned above were determined after a single dose and every 7 days throughout the experimental period. It was found that the extract at all dosage range used in this study significantly decreased escape latency at day 7 of treatment. In addition, the results also showed that rats subjected to medium and high doses of treatment showed increased retention time. No other significant changes were observed. Our results suggest that *M. oleifera* possesses anti-oxidant and cognitive enhancing effects. *M. oleifera* may thus be a beneficial neuroprotectant and cognitive enhancer; however, more research is needed to elucidate this issue.

Keywords: *Moringa oleifera Lam, phenolic compound, anti-oxidative, neuropharmacological activities*

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O-14

Effect of phytoestrogen from *Curcuma comosa* Roxb. on Wnt/β-catenin signaling pathway

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Wnt/β-catenin signaling pathway plays crucial roles both in normal development and in diseases, including cancer. Recently, a growth promoting effect of estrogen in many target cells has been reported to activate Wnt/β-catenin signaling pathway, which is a rapid non-genomic action of estrogen. *Curcuma comosa* Roxb. (Wan chak motluk) which contains phytoestrogens and is widely used as an alternative supplement for treatment of unpleasant symptoms in menopausal women. However, it is not clear whether these phytoestrogens act in a similar manner to that of estradiol. In the present study, we aims to investigate the mechanistic effect of diarylheptanoid compound-049 which has been found to exhibits the highest estrogen-like activity on Wnt/β-catenin signaling pathway. By using a TOPflash luciferase assay in HEK 293 cells, treatment with compound 049 rapidly activated β-catenin-mediated Wnt signaling and induced a significant increase in β-catenin protein levels similar to those of estradiol. Moreover, immunofluoresence in CHO cells demonstrated that β-catenin was predominantly localized in the nucleus when cells were treated with estradiol and compound-049. The phosphorylation at serine 9 of GSK-3β was increased and inhibited its activity in cells treated with estradiol and compound-049. This result indicated that the activation of Wnt/β-catenin signaling mediated by estradiol and compound-049 is dependent of glycogen synthase kinase-3β (GSK-3β) the negative regulator of Wnt/β-catenin signaling pathway. Finally, compound-049 also increased the expression of β-catenin in mouse osteoblastic cells (MC3T3-E1). These results suggest that both estradiol and compound-049 exhibits a rapid non-genomic action by activating GSK-3β-dependent Wnt/β-catenin signaling pathway. Importantly, our results demonstrate the mechanistic effects of phytoestrogens from *Curcuma comosa* Roxb. on Wnt/β-catenin signaling pathway for the first time and is considered as the important information required for further development of this natural compound as the supplement material for post-menopausal women.

Keywords: *Curcuma comosa, 17β-estradiol, β-catenin, GSK-3β, Wnt signaling*

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P-01

Inhibition of L-type amino acid transporter 1 (LAT1) suppresses cholangiocarcinoma cell growth

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Cholangiocarcinoma (CCA) is a malignant tumor arising from bile duct epithelial cells, associated with liver fluke (*Opisthorchis viverrini)* infection. CCA is a rare tumor worldwide but highly prevalent in northern and northeastern Thailand. Chemotherapeutic treatment of CCA is ineffective; therefore, new molecular targets for treatment are required. The L-type amino acid transporter 1 (LAT1) is known to upregulate the plasma membrane of several types of tumor cells, however, its role(s) in CCA cell growth has not been reported. We, therefore, evaluated the impact of LAT1 on CCA cell proliferation and investigated LAT1 expression and its transport activities in human CCA cells (HuCCA-1). Real-time PCR analyses demonstrated that HuCCA-1 cells expressed LAT1 together with its associated protein 4F2 heavy chain (4F2hc). By immunoblotting analyses, using the anti-LAT1 antibody, we detected the band at MW 125 kDa, which corresponds to the heterodimeric complex of LAT1 and 4F2hc cleaved to MW 37 kDa, the original size of LAT1 monomer in the presence of DTT. This result indicates that LAT1 formed a functional complex with 4F2hc in HuCCA cells. In addition, LAT1 mediated Na^+-independent [^{14}C]L-Leucine transport and was inhibited by the system L selective inhibitor BCH (2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid). The inhibition of LAT1 by BCH in HuCCA cells resulted in a reduction of phosphorylation of p70S6 kinase (a down-stream effector of mTOR) and suppressed their proliferation. Our findings indicate that LAT1 is required in order to provide the essential amino acids to HuCCA cells for continuous growth and proliferation. Inhibition of LAT1 suppresses cancer cell growth; thus, LAT1 appears to be a promising target for cancer therapy.

Keywords: *L-type amino acid transporter 1, Cholangiocarcinoma, BCH, mTOR*

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P-02

Passiflora foetida attenuates brain ischemic volume and oxidative stress in animal model of focal cerebral ischemia

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Oxidative stress is reportedly implicated in various pathological conditions including stroke. The therapeutic strategy against this condition is currently very limited; however, the paradigm shift to prevention (health promotion) over against treatment should encourage the development of food supplements to protect against this condition. We aimed to determine the effect of *Passiflora foetida* extract on the brain ischemic volume and oxidative damage markers. Male Wistar rats were given the extract of the aerial part of *P. foetida* orally at 25, 100 and 400 mg/kg BW, then the right middle cerebral artery (Rt. MCAO) was occluded. The brain ischemic volume and oxidative damage markers of the rats were determined 24 hr after Rt. MCAO. At all doses of the extract used in this study brain ischemic volume in cortical area was attenuatedwhile only the low dose attenuated ischemic volume in the subcortical area. A marked reduction of the oxidative damage marker, malondialdehyde(MDA) level, was also observed. The current data suggest that the neuroprotective effect of *P. foetida* might occur partly via antioxidant activity.*P . foetida* has potential as a food supplement to protect against stroke, so further researchis necessary.

Keywords: *Passiflorafoetida, cerebral ischemia, focal stroke, oxidative stress*

Acknowledgements: This study was supported by the North-eastern Stroke Research Group and Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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P-03

Moringa oleifera Lam. leaves extract improves the function of nerve in experimental diabetic neuropathy

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Moringa oleifera Lam., a popular herb from a family of Moringaceae, is reputed to have anti-diabetic, anti-inflammation and anti-oxidant activities. Recent findings showed that these activities were implicated in the functional retardation of diabetic neuropathy. This study was therefore undertaken to determine the effect of *M. oleifera* leaf extract on the function of the sciatic nerve in an experimental model of diabetic neuropathy, induced by chronic constriction of the sciatic nerve in diabetic rats. Male Wistar rats, weighing 200-250 g, had diabetes mellitus induced using streptozotocin (STZ) then diabetic neuropathy induced by permanent ligation at the upper one-third of the right sciatic nerve. The rats were then given the plant extract orally at doses of 100, 200 and 400 mg/kg BW once daily for 14 days. The animals had the function of sciatic nerve assessed using the sciatic function index (SFI) from De Medinacelli. The sensory withdrawal latencies were determined using both the hot plate and Von Frey filament tests. The results showed that after the 14-day treatment period, rats subjected to the *M. oleifera* treatment at all dosages had a significantly improved SFI and reversed elevation of sensory withdrawal latencies to both thermal (noxious) and touch (non-noxious) stimuli induced by the constriction at the sciatic nerve. However, only the medium dose treatment group showed any significant changes in SFI and sensory withdrawal latency to thermal stimuli. These data suggest that *M. oleifera* improved sciatic nerve function in a diabetic neuropathy animal model. *M. oleifera* could therefore be beneficial for those suffering from diabetic neuropathy. Understanding the precise underlying mechanism requires further investigation.

Keywords: *Moringa oleifera Lam., diabetic neuropathy, functional recovery of sciatic nerve*

Acknowledgements: A financial support for this research was provided by the Food and Functional Food Cluster under the National Research University Program of Khon Kaen University and the Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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P-04

Anti-inflammatory activity of Thai traditional remedy Sahasthara extracts for treatment of muscle pain

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The Sahasthara remedy (ST) comes from the Ayurvedic College, Thailand. It is prepared from the parts of 21 plant species including, *Acorus calamus* Linn.(rhizome), *Anacyclus pyrethrum* (L.) DC.(root), *Anethum graveolens* L. (seed), *Atractylodes* lancea (Thunb.) DC. (root), *Baliospermum montanum* (Willd.) Muell. Arg. (root), *Cuminum cyminum* L. (seed), *Cinnamomum camphora* (L.) J.S. Presl *Ferula assafoetida* L.(oleo gum resin), *Kleinhovia hospita* L. (root), *Lepidium sativum* L. (seed), *Nigella sativa* L. (seed), *Merremia vitifolia* (Burm.f.) (root), *Myristica fragrans* Houtt. (aril and seed), *Pimpinella anisum* L. (Seed), *Picrorrhiza kurroa* (root), *Piper nigrum* Linn. (fruit), *Piper retrofractum* Vahl. (fruit), *Plumbago indica* Linn. (root), and *Terminalia chebula* Retz. (fruit and gall). These plants were examined for their inhibitory activities against lipopolysaccharide (LPS) including nitric oxide in RAW 264.7 cell lines. The results indicate that the ethanolic extract of ST exhibited the most potent inhibitory activity against nitric oxide production (IC_{50} = 2.64±0.19 μg/mL), followed by the ethanolic extract of *Terminalia chebula* Retz., *Baliospermum montanum* (Willd.) Muell. Arg., *Piper retrofractum* Vahl., *Atractylodes* lancea (Thum.) DC., with IC_{50} of 9.70±0.54, 12.55±1.60, 25.90±2.5, 28.70±3.3 μg/mL, respectively. These results support the traditional folklore assertion that Sahasthara is a remedy for inflammatory-related diseases and we now know this achieved though the inhibition of NO release.

Keywords: *Sahasthara, anti-inflammation, inflammation, nitric oxide, antioxidant, COX_2, TNF-α*

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P-05

Deferiprone alleviates oxidative stress in mice with iron overload condition

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Iron is the most abundant metallic microelement in the body and essential for erythropoiesis. However, excess iron in the body can lead to iron overload and increasing production of free radicals. It is reported that long-term consequences of iron toxicity are preventable and mostly reversible by effective iron chelation therapy. Deferiprone is the low molecular weight oral iron chelator which is widely used in patients with beta-thalassemia major. In this study, we hypothesized that deferiprone could reduce oxidative stress in mice with iron overload condition. Male ICR mice were induced iron overload by intraperitoneal injection of iron sucrose (10 mg/kg/day), three days/week for eight weeks. Deferiprone at dose of 50 mg/kg was intragastrically administered once daily for five days per week throughout the period of iron sucrose administration. After eight weeks, arterial blood pressure and oxidative stress markers were evaluated. A marked increase in oxidative stress and arterial blood pressure was found in mice received iron sucrose. Treatment with deferiprone ameliorated these effects by restoring the blood pressure, decreasing vascular superoxide production, and reducing malonaldehyde levels in both plasma and tissues as compared to those found in the control animals ($P<0.05$). Results in this study suggest the beneficial effect of deferiprone on reducing vascular dysfunction and oxidative stress in iron overload condition.

Keywords*: iron overload, iron sucrose, deferiprone, oxidative stress*

Acknowledgments: Deferiprone was a generous gift given by the Government Pharmaceutical Organization of Thailand. Weerapon Sangartit was supported by the Royal Golden Jubilee Ph.D. program, The Thailand Research Fund.

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P-06

Aortic-femoral pulse wave velocity may be used as a predictor of coronary artery disease severity in the metabolic syndrome patients

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Metabolic syndrome (MetS) is characterized by multiple risk factors and is associated with increased risk of coronary artery disease (CAD). Recently, arterial stiffness and CAD have been shown to be closely related, but data on the correlation between the severity of CAD and arterial stiffness, especially in MetS patients is very limited. Pulse wave velocity (PWV) is widely recognized as a good marker of arterial stiffness and is now known to be a good prognostic indicator for cardiovascular disease. The aim of this study is to evaluate whether an increase in PWV is associated with the severity of CAD in Thai MetS patients. One hundred and fifty-three patients with mean age of 61.5 ± 0.6 years were enrolled in this study. Routine anthropometric and serologic data were collected. PWV was measured the day before coronary angiography, and the severity of CAD was assessed using the modified Gensini scoring system after angiography. One hundred and eleven patients were classified as MetS according to the National Cholesterol Educational Program-Adult Treatment Panel III (NCEP-ATPIII) and International Diabetes Federation (IDF) criteria. Seventy-three patients with MetS were diagnosed CAD after angiography. Aortic-ankle PWV (aaPWV) and aortic-femoral PWV (afPWV) were significantly increased in MetS patients. The modified Gensini score was significantly correlated to afPWV (r=0.235, p=0.016) after adjustment for factors know to influence PWV, including age, sex, systolic pressure, ankle-brachial pressure index, fasting blood sugar, and high density lipoprotein-cholesterol. Our findings suggest that afPWV, as an established measure of central artery stiffness, may be used as a screening tool for predicting the severity of CAD, especially in patients with metabolic syndrome.

Keywords: *central arterial stiffness, coronary artery disease, Gensini score, metabolic syndrome, pulse wave velocity*

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P-07

Anacardium occidentale leaf extract enhances sexual function in stress exposed rats

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The leaves of *Anacardium occidentale* (family Anacardiaceae) have long been used in the daily diet and as an aphrodisiac in Southern and Northeastern Thailand. Despite its reputation and longstanding usage, there is to our knowledge no scientific assessment of these claims. This study was conducted to determine the effect of *A. occidentale* leaf extract on the sexual function of male rats subjected to stressors. Male Wistar rats, 8 weeks old, weighing between 200 and 220 g, were given *A. occidentale* leaf extract orally at doses of 25, 100 and 200 mg/kg BW, 45 minutes prior to a 12-hr exposure to immobilization stress. After 3 hr of refreshing time, they were paired with sexually receptive females. We then determined mounting latency, mounting number, intromission latency, intromission number, ejaculatory latency and ejaculatory number, after the single dose administration and every week throughout the 2-week experimental period. The results showed that at 7 days of treatment, the rats which received *A. occidentale* leaf extract at a dose of 100 mg/kg BW demonstrated decreased latency in mounting, intromission and ejaculation and increased frequency of mounting and intromission. There was, however, no significant change in ejaculation number. This is the first study to confirm the aphrodisiac activity of *A. occidentale*as per traditional folklore. *A. occidentale* leaf extract has potential as a functional food or nutraceutical, but further research for extension to humans is needed.

Keywords: *Anacardium occidentale, stress, sexual behaviors*

Acknowledgement: This study was supported by the National Research Council of Thailand and Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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P-08

Antihypertensive effect of peptides-derived from byproduct of rice bran oil manufacture in two kidney-one clip renovascular hypertensive rats

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Rice (*Oryza sativa*) is not only the staple diet of the Thai people, it is also the most important export product of the country. Jasmine rice or Thai Hom Mali rice is the most popular cultivar of rice grown in Thailand. Rice bran protein hydrolysates extracted from rice have been reported to be a rich source of bioactive peptides. Based on a recent report on rice bran peptides (RBP), derived from byproduct of rice bran oil manufacture, antioxidant and angiotensin converting enzyme inhibitory properties have been demonstrated in an *in vitro* study. Since there is limited evidence of the biological activity of RBP in lowering blood pressure, we therefore aimed to evaluate the antihypertensive effect of RBP in two kidney-one clip (2K-1C) hypertensive rats. Male Sprague-Dawley rats had 2K-1C hypertension induced by placing a silver clip (0.2 mm i.d.) around the left renal artery, whereas sham-operated rats served as the controls. After six weeks, 2K-1C rats with systolic blood pressure (SBP) > 160 mmHg and sham-operated rats with normal SBP were intragastrically administered RBP (500 mg/kg/day) or vehicle for four weeks. At the end of the study period, the mean arterial pressure of 2K-1C rats treated with RBP was significantly lower than those of untreated 2K-1C rats ($P<0.01$). Moreover, RBP also increased hindlimb blood flow and decreased hindlimb vascular resistance in 2K-1C rats, suggesting the reduction in blood pressure might be due to the vasodilating effect of RBP. Regarding the antioxidant activity, we found a significant decrease in superoxide production in the carotid arteries of 2K-1C rats treated with RBP. Thus, the peptides-derived from byproduct of rice bran oil manufacture could serve as a functional nutrient against hypertension and oxidative stress.

Keywords: *Thai rice bran peptides, 2K-1C hypertension, oxidative stress*

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P-09

Does perivascular cholinergic nerve innervate rat mesenteric arteries?

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The peripheral organs and tissues are innervated by autonomic nerves (sympathetic adrenergic and parasympathetic cholinergic nerves). However, the vascular blood vessels have been shown to be innervated only by sympathetic adrenergic nerves. The rat mesenteric arteries have been shown to be innervated by various perivascular adrenergic and non-adrenergic nerves, while functional cholinergic innervation remained unknown. Therefore, we designed to investigate pharmacologically the functional cholinergic innervation by using cholinergic agents (neostigmine, atropine, hexamethonium) in rat mesenteric arteries. The rat mesenteric vascular beds without the endothelium were isolated and prepared for perfusion to measure perfusion pressure. In preparations treated with capsaicin (CGRP depletor) or in the presence of L-NAME (nitric-oxide synthase inhibitors), atropine, hexamethonium and neostigmine had no significant effect on contractile responses to perivascular stimulation (2-12 Hz) and exogenously applied noradrenaline (NA). Neurogenic release of NA in the perfusate was not affected by cholinergic agents used. Immunohistochemistry study of the mesenteric artery showed choline acetyltraqnsferase-immunopositive fibers, which were resistant to 6-hydroxydopamine (chemical adrenergic toxin) and capsaicin. These results suggest that the rat mesenteric artery has cholinergic innervation, which is different from adrenergic and capsaicin-sensitive nerves and is not associated with vascular tone regulation.

Keywords*: cholinergic innervation, perivascular nerves, rat mesenteric artery*

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P-10

Quercetin-loaded zein-based nanofiber mat: a novel cognitive enhancer

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Cognitive function is a key determinant of memory; hence the search for effective and potent cognitive enhancers. Based on previous knowledge quercetin can enhance memory and increase drug delivery by nanofibers. We thus hypothesized that quercetin-loaded nanofibers could effectively enhance memory performance and decrease neurodegeneration. We evaluated the novel zein-based nanofiber mat, loaded with quercetin, on memory performance and on the survival of neurons in the hippocampus of rats. Quercetin-loaded zein-based electrospun nanofiber mats at concentrations of 10 and 15% were successfully prepared: quercetin was gradually released from the fiber mats. The mats were applied on the dorsal of healthy rats for 14 days. The spatial memory using Morris water maze test and neuron density in the hippocampus was determined at the end of the experimental period. Oxidative markers in hippocampus (including MDA, SOD, CAT and GPx) were also determined. The results showed that quecetin-loaded zein-based nanofiber mats enhanced memory and neuron density in the hippocampus of healthy rats. The possible underlying mechanism might be associated with its antioxidant activity which might decrease MDA levels and increase SOD activity in the hippocampus. Quercetin-loaded zein-based nanofiber mat have potential as a novel cognitive enhancer.

Keywords: *antioxidant, quercetin, brain, transdermal drug delivery, Nanofibers*

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P-11

Anticholinesterase activity of constituents of essential oils from Thai medicinal plants

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A previous study found that tumeric, galanga and fingerroot oil had strong anticholinesterase activity. The active constituents of these oils were determined by TLC bioautography and gas chromatography-mass spectrometry (GC-MS): (a) trans-caryophyllene, 1,8-cineole and camphene of turmeric oil (b) 1,8-cineole, terpinen-4-ol and methyl eugenol of galanga oil and (c) 1,8-cineole, methylcinnamate, camphor, linalool and geraniol of fingerroot oil. The anticholinesterase activities of active constituents of each oil were examined. Owing to the solubility limit of the constituents, anticholinesterase activity was reported in terms of %inhibition value at a concentration of 50 μg/mL. Trans-caryophyllene, *the most active constituent of tumeric oil (% inhibition value of 54.81±0.97),* showed a % inhibition value of 33.01±2.92 while 1,8-cineole *the most active constituent of both galangal (% inhibition value of 52.13±0.47)* and had a % inhibition value of 17.83±0.63. These results indicate that all of these oils *fingerroot oil (% inhibition value of 46.9±1.21)* had a stronger activity than their active constituents**.** Additionally, the anticholinesterase activities of the combination between the active constituents of each the oils were determined. The ratio between the active constituents used in this study was according to the result from GC-MS. An additive, synergistic as well as the inhibitory effect between the active constituents was also found. These findings revealed that the anticholinesterase activity of the oils may be the result of the interaction between their active constituents and from other constituents in the oil.

Keywords: *constituents, essential oils, Thai medicinal plants, anticholinesterase activity, % inhibition*

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P-12

Protective effect of shallot (*Allium ascalonicum*) on paracetamol induced hepatotoxicity in mice

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Overdose of paracetamol causes hepatotoxicity by its toxic oxidation metabolites. Shallot (*Allium ascalonicum*) is rich in phenolic compounds and showed high antioxidant properties. An objective of this study is to evaluate the protective effect of *A. ascalonicum* against paracetamol induced hepatotoxicity. Thirty male mice strain IRC were allocated into 5 groups, 6 mice for each, group I received distilled water 0.5 ml/100 gBW as negative control, group II received paracetamol 0.5 g/100 gBW as positive control, group III received *A. ascalonicum* juice at dose of 1.0 g./100 gBW as extract toxicity test, groups IV and V received *A. ascalonicum* juice at dose of 0.5 and 1.0 g/100 gBW, respectively for 4 days and co-treated with paracetqmol 0.5 g/100 gBW for 14 days as treated groups. All groups were determined serum alanine aminotransferase (ALT) and examined histological changes of liver. Serum ALT of paracetamol treated group was increased in 3 times than those of the control group and was supported by high incidence of liver lesion. Both groups pretreated with *A. ascalonicum* juice before co-treated with paracetamol showed decreasing of serum ALT and percentage of liver lesion. It could be suggested that *A. ascalonicum* juice may be used for attenuation the hepatotoxicity induced by paracetamol.

***Keywords**: paracetamol, Allium ascalonocum, hepatotoxicity, protective effect*

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P-13

Anacardium occidentale leaves extract enhances muscle performance

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Intense or prolonged exercise can produce free radicals and induce radical-mediated injury to skeletal muscles, particularly in untrained individuals. Radicals probably contribute to muscular fatigue during endurance events; thus, the development of ergogenic food supplements from substances possessing antioxidants has gained much concentration. Thus, the purpose of the current study was (a) to determine the effect of the extract of *Anacardium occidentale* leaves on muscle performance and endurance and (b) to explore the possible underlying mechanisms of the extract on both muscle fiber size and density. Male mice, weighing between 30 and 50 g were given the extract orally for 4 weeks then anti-fatigue, muscle endurance and maximum force of contraction were determined. The results indicated that mice given the extract (a) took longer to develop fatigue and (b) had enhanced the endurance capacity and muscle contraction capacity. The optimum dose was 100 mg/kg BW. The underlying mechanism occurred partly via the increased muscle fiber size and decreased oxidative stress. Although further research is required, it appears that the extract of *A. occidentale* leaves has potential to be developed as a health product for athletes and laborers for enhanced muscle performance.

Keywords: *Anacardium occidentale, endurance, anti-fatigue, muscle performance*

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P-14

Anti-lipid peroxidation and antioxidant activity of *Polygonum odoratum* Lour.

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Oxidative stress has been implicated in the pathology of a variety of human diseases. A potential therapeutic intervention may include natural antioxidants. The antioxidant activity of *Polygonum odoratum* Lour. (POL) known locally as "Pug Peaw" was observed in previous *in vitro* and *in vivo* studies. The ethanol extract of POL (dried whole plant) has scavenging activity with the stable free radical DPPH and antioxidant activity, observed as protecting rat liver from acetaminophen-induced injury. The current study aimed to examine the anti-lipid peroxidation and antioxidant activity of POL extracted by various solvents (with different polarity), namely; chloroform, petroleum ether, ethyl acetate and methanol. The ethanol extract of POL proffered significant antioxidant activity by acting as an inhibitor of lipid peroxidation in the membrane of rat liver microsome induced by iron-ascorbic-H_2O_2 (TBARs method) in a dose-dependent manner (IC_{50} of 115 mg/ml). The POL strongly inhibited the auto-oxidation of linoleic acid by decreasing production of iron thiocyanate complexes. At a concentration of 30 mg/ml, the extract showed complete inhibition of auto-oxidation. An additional study of POL extracts using two *in vitro* tests and various solvents were done to test antioxidant activities: firstly, by determining scavenging on DPPH; and, secondly, by Ferric Reducing/Antioxidant Power assay (FRAP). The results indicated that the POL extracts from all of the solvents have dose-dependent, antioxidant activity. Ethyl acetate and methanol were more effective extracts than chloroform and petroleum ether. Taken together, it was concluded that the active ingredients of POL are polar in nature, so have better antioxidant activity.

Keywords: *antioxidant activity, lipid peroxidation, Polygonum odoratum Lour., solvents*

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P-15

Acute effects of cashew juice on oxidative stress and immune function after intense exercise in sedentary subjects

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Vitamin C is one of the most powerful antioxidants. It can also improve immune function among humans. Juice from the cashew apple contains a high level of vitamin C; thus, it may enhance the functioning of the immune system. Confirmation and/or demonstration of any favorable effects by cashew apple juice on the immune function are not known. The present study, therefore, aimed to investigate acute effects of the cashew apple juice on immune function in sedentary subjects. This study used a randomized crossover design. Ten healthy men between 18 and 45 years of age were enrolled. They randomly ingested either cashew apple juice or a placebo 3.5 ml/kg body weight before cycling at intensity of 85% of maximal oxygen consumption ($\dot{V}O_{2,max}$) for 20 minutes. Plasma vitamin C, neutrophil and lymphocyte functions were determined at rest (T0), at 20 min after ingestion (T20), after the exercise (T40), and after 30 min of recovery (T70). Plasma vitamin C was highest at T20 after ingestion of the cashew apple juice ($p<0.05$) which then gradually decreased. At T20, T40 and T70, the plasma vitamin C was higher in subjects supplemented with the cashew apple juice compared to those given the placebo ($p<0.05$). However, the neutrophil and lymphocyte functions were not significantly different between groups. These results indicate that a single dose ingestion of the cashew apple juice contributed to an increase in body antioxidants by increasing plasma vitamin C, but without any difference in immune function. This lack of any measureable impact on immune function could be due to the short-term duration of supplementation.

Keywords: *cashew apple juice, oxidative stress, vitamin c, antioxidant, immune function*

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Vasodilatation effect of *Mesua ferrea* Linn. extract on isolated rat aortas

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Mesua ferrea Linn. belongs to the family Guttiferae. It is known as "Bunnak" in Thailand. In traditional medicine, its flowers are used as a heart tonic, astringent, stomachic and expectorant. The flower buds are used for dysentery. The main chemical constituents include coumarins, saponins, phenols and alkaloids. The vasodilatation effect of the 95 % ethanolic extract on isolated rat aortas was investigated. The result showed that the flowers extract (10, 200 and 400 μg/mL) could reduce muscle tone in a respective 29.3 %, 33.6%, 42.0% of isolated endothelium-intact thoracic aortic rings pre-constricted with phenylephrine (10^{-6} M), except for the endothelium denuded rings. The results indicate that the vasorelaxation effect of the extract of *Mesua ferrea* (Bunnak) flowers depended on its concentration and the aortic endothelium.

***Keywords**: Mesua ferrea Linn., vasodilatation, ethanolic extract*

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Cardioprotective effect of Jasmine flower extract on myocardial infarction rats

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The cardioprotective effect of an ethanolic extract from *Jasminum sambac* (L.) Ait. 'G. Duke of Tuscany' flowers **was** investigated in rats with isoproterenol-induced myocardial ischemia. Three groups of Wistar rats (5 per each group) were used: Group 1 served as the control; Group II were given water for 28 consecutive days and isoproterenol (100 mg/kg, i.p.) twice at an interval of 24 h on the 29th and 30th day; and, Group III were given extract (250 mg/kg body weight) once a day for 28 consecutive days and isoproterenol (100 mg/kg, i.p.) twice at an interval of 24 h on the 29th and 30th days. The cardiac marker enzymes were determined, including: *aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) in plasma*. The results indicate that isoproterenol significantly increases CK, LDH and the alanine aminotransferase in plasma. Pretreatment with an ethanolic jasmine flower extract had an effect on the activity of marker enzymes near the norm, indicating a cardioprotective effect in rats by the extract of *Jasminum sambac* flowers against isoproterenol-induced biochemical changes.

Keywords: *Jasminum sambac (L.) Ait. 'G. Duke of Tuscany',* cardiac marker enzymes, myocardial infarction, isoproterenol

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Effect of tomato pomace on high fat diet induced obesity rat

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The increased prevalence of obesity worldwide and its impact on lifestyle-related diseases, such as metabolic syndrome stroke and cancer, require novel strategies. Despite the urgent need for safe and effective therapeutic strategies and the potential market for anti-obesity food supplements, the current status of food supplements targeting obese personsremains inadequate. The current study thus aimed to test the effect of tomato pomace, a high fiber and high phenolic compound substance, on a high fat diet. Male Wistar rats had obesity induced using a high fat diet and were treated with various doses of tomato pomace (2,10 and 50 mg/kg BW) for 45 days. We then determined weight gain, food and water intake, the fat pad, and the alteration of adipocyte and triglyceride level.The results showed that the obese rats which received tomato pomace had less weight gain than the non-treated group as well as decreased plasma triglyceride and adipocyte diameter. The underlying mechanism for ant-obesity effect of tomato pomace might be that tomato waste powder decreases plasma triglyceride levels, resulting in a reduction of triglyceride deposition in adipocytes. Tomato pomace appears to provide abeneficial effect which mitigates obesity and its associated lifestyle-related diseases.

Keywords*: tomato pomace, obesity, high fat diet, adipocyte*

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Subchronic toxicity of alcoholic *Anacardium occidentale* L. leaf extract

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Anacardium occidentale L. leaves have long been used as food in Southern and Northeast Thailand. Recent reports showed the therapeutic potential of *A. occidentale* leaves, including as an analgesic, anti-inflammatory, anti-diarrheal and anti-oxidant. This study was carried out to determine the pre-clinical activity and safety profile of *A. occidentale* leaf extract. The lyophilized alcoholic extract of *A. occidentale* leaves was administered orally at doses of 20, 100 and 500 mg/kg BW daily for 100 days to Wistar rats. Selected hematological and biochemical parameters were determined at the end of the experiment. The results showed a difference according to sex in vulnerability to the extract. While the NOAEL (no-observed adverse effect level) in males was 20 mg/kg BW, we did not find a NOAEL effect in females. Instead the LOAEL (lowest adverse effect level) was 20 mg/kg BW. Thus, the subchronic administration of *A. occidentale* leaf extract did not produce significant toxic effect in males. This is, to our knowledge, the first report that *A. occidentale* leaf extract is safer for male rats than female rats. Testing of *A. occidentale* leaf extract as functional food or nutraceutical should focus on its use in males. Further investigation is necessary.

Keywords: *Anacardium occidentale leaves, leaf extract, subchronic toxicity, NOAEL, LOAEL*

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Acute toxicity effect of *Lycopersicon escalentum* extract in mice

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Phytomedicine has long been used in traditional medicine. Although it is generally agreed that medicinal plants and their products are naturally safer than synthetic drugs. A general assumption regarding safety should not be made without testing. Before stating the safety and therapeutic index of the medicinal plant extracts, the acute toxicity of the plant extract should be tested. This study was carried out to determine the acute toxicity of *Lycopersicon escalentum* extract in mice, following oral administration of graded doses of the plant extract. The results revealed the mice showed no signs of depression, unsteady gait, tremors, and respiratory distress and no death with doses up to 30 g/kg body weight. This suggests that *L. escalentum* extract is safe for oral administration and use as a functional food or nutraceutical; however, further studies are also required, especially regarding subchronic and chronic toxicity.

Keywords: *Lycopersicon escalentum, acute toxicity*

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Acute toxicity test of *Anacardium occidentale* leaf extract

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The use of herbal health products has increasing acceptance worldwide. The leaves of *Anacardium occidentale* L., a medicinal plant in the Anacardiaceae family, are said to have various medicinal purposes. Scientific data about their activity and safety test are, however, not well known. The current study aimed to determine the acute toxicity of alcoholic of *A. occidentale* leaf extract in both mice and rats. Wistar male and female rats and mice, weighing between 180 and 200 and 30 and 40 g, respectively, were given the extract orally at doses of 100, 500 and 2000 mg/kg BW. The results revealed no deaths with doses up to 2000 mg/kg BW. At the highest dose, there were no signs and symptoms indicating toxicity within 24 h of extract administration. After 14 days follow-up, all of the animals still showed no signs and symptoms of toxicity. At the end of experiment, the histopathological data obtained from necropsy confirmed that there was no toxicity in either the males or females of both species. Our findings demonstrate that alcoholic *A. occidentale* leaf extract is not acutely toxic. This study provides a safety range for oral dosing that can be used in further research.

Keywords*: Anacardium occidentale leaf, acute toxicity*

Acknowledgements: This study was supported by the National Research Council of Thailand and Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.

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Biological activities of waste product from *Antidesma thwaitesianum* Müll.Arg

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The fruit of *Antidesma thwaitesianum* is used as a popular soft drink, wine and jam. The waste product or residue after squeezing of the fruit was investigated for its biological activity, such as: antimicrobial and antioxidant activities**.** The dried mash from squeezing were collected and prepared for three different extraction methods (*viz.*, decoction, maceration with 95% EtOH and decoction of residue of maceration W, E and EW, respectively). All of the extracts were assayed for antimicrobial activity using disk diffusion assay, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The extracts were investigated against representative gram-positive (*Staphylococus aureus* and *Bacillus subtilis*) gram-negative (*Escherichia coli* and *Salmonella typhi*) and fungi (*Candida albicans* and *Cryptococcus neoformans*). Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Total phenolic content was also determined using the Folin-Ciocalteu reagent. EW and W showed the strongest DPPH scavenging activity (EC_{50} 11.73±0.52 and 19.49±0.24μg/ml, respectively). EW was related to the total phenolic content as it showed the highest value of phenolic content as 61.37±0.24 mg GAE/g dw. All of the extracts showed inhibited activity against *S. aureus*. Both aqueous extracts (*i.e.*, W and EW) showed the highest antimicrobial activity on the disk diffusion assay; they produced a 8-mm inhibition zone. Only the ethanolic extract (E) exhibited antimicrobial activity against *B. subtilis*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of extracts were also determined against *S. aureus and B. subtilis*. The extracts from the waste of *A. thwaitesianum* showed less antibacterial potential. On the other hand, its decoction residue of the maceration extract (EW) showed high antioxidant power and the highest phenolic content. Thus, the post-squeezing waste product of this fruit could be useful for health and should be recommended as a source of natural antioxidants.

Keywords: *Antidesma thwaitesianum, antibacterial, antioxidant, total phenolic content*

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Mutagenicity and anti-mutagenicity of *Erythrophleum succirubrum* extract

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Erythrophleum succirubrum (*Gagnep* or *Phan Saat* in Thai) is a toxic plant belonging to Leguminosae-Caesalpinioideae family. Ingestion of any part of this plant will cause vomiting, diarrhea, cardiac arrest and death. Notwithstanding, it is used in a Thai traditional medicine for treatment of fever. In this study, the ethanolic extract of *E. succirubrum* stems was investigated for its mutagenicity and anti-mutagenicity using a bacterial model, I*Salmonella typhimurium* strain TA98 and TA100), based on the Ames' test. The result showed that the extract, at concentrations between 1 and 10 mg/plate, had no mutagenicity in the bacterial model in the presence and/or absence of enzymatic activities (S9 mix). In the absence of the S9 mix and addition of the standard mutagen (2-2 furyl-3-(5-nitro-2 furyl) acrylamide or 4-nitroquinoline-1-oxide), the extract showed anti-mutagenicity in the respective bacteria strain TA98 and TA100 with 50% inhibitory concentrations (IC_{50}) of 5.30 and 4.74 mg/plate and 6.76 and 4.55 mg/plate. The addition of the standard mutagen 2-amino anthracene in the presence of S9 mix, the extract showed strong anti-mutagenicity in the bacteria strain TA98 and TA100 with IC_{50} at 0.63 and 0.63 mg/plate, respectively. These results suggest that the extract had anti-mutagenicity in the presence and absence of enzymatic activities; especially the metabolized form of the extract that exhibited strong activity at very low concentrations in a dose-dependent manner. The *E. succirubrum* extract itself did not induce mutation but the metabolized form could reduce mutagen-induced mutations in this bacterial model.

Keywords: *Erythrophleum succirubrum, mutagencity, anti-mutagenicity, Ames' test*

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Nitric oxide inhibitory effect of the extract from *Musa sapientum* Linn.

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Thai traditional medicine uses many parts of the plant *Musa sapientum* Linn. (Musaceae) in drugs, cosmetics and as a health food. The unripe fruit were used as an anti-diarrheal and the ripe fruit as a tonic. The peel of the fruit was used for making a foot cosmetic and for wound treatment. The objective of this research was to investigate the anti-inflammatory activity by determing the inhibitory effect on nitric oxide production on RAW264.7 cells stimulated by lipopolysaccharide (LPS) from each part of the banana. Cytotoxic activity, by MTT assay, against the RAW264.7 cell was tested to determine the concentration inhibiting NO release prior to cell death. Macerated fresh and dry, ripe and unripe peel of *M. sapientum* were extracted with 95% ethanol, soaked and boiled in water or decoction. We found that the water extract by soaking fresh, unripe fruit showed the highest anti-inflammatory activity, with an IC_{50} of 6.68 μg/mL, followed by the ethanolic extracts of unripe peel (IC_{50} values of 36.62 μg/mL). The fresh ripe peel of *M. sapientum* Linn. has potential as a source of natural anti-inflammatory ingredients and therapeutic value in Thai traditional medicine.

Keywords: *Musa sapientum, anti-inflammation, nitric oxide inhibition assay, RAW264.7 cell*

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Effects of broccoli on operant conditioning, behavior-related learning and memory in rats

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Broccoli (*Brassica oleracea* L.) contains a variety of vitamins, especially folic acid which helps nourish the nervous system. The procedure of conditional discrimination has been used to study learning, memory and perception. Learning and memory in rats were studied using the operant conditioning technique, which assesses the likelihood of a behavior being increased or decreased through reward and/or punishment. The objective of this study was, therefore, to determine whether broccoli could improve learning and memory impairment induced by scopolamine via operant conditioning. Rats were orally administered 250, 500 and 750 mg/kg broccoli powder (BP) for four days. They were then injected with 0.5 mg/kg scopolamine. A 30-min session for operant acquisition was introduced to the rats. The results showed that those given 750 mg/kg BP exhibited the highest scores of lever pressing in the 2nd and the 3rd cycles of FR condition and in the 1st and the 2nd cycles of VR condition. Those given 750 mg/kg BP also had low scores for wrong lever pressing, especially in the 2nd and the 3rd cycles. These findings suggest that BP may have a cognitive improvement quality involving the cholinergic pathway.

Keywords: *Brassica oleracea L., learning, memory, operant conditioning*

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A novel polyphenolic compound derived from shells of mangosteen targets three distinct Cl^- channels in human intestinal cells: the physiological basis for antidiarrheal applications of mangosteen

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Secretory diarrhea is caused by enterotoxin-induced intestinal Cl^- secretion with cAMP or Ca^{2+} being the secondary messengers. Shells of mangosteen have long been used as a traditional medicine for diarrhea in Thailand. The active ingredients together with mechanisms underlying this therapeutic benefit remain unexplored. Phenol-containing compounds are abundant in the shells of mangosteen; therefore, the aim of the present study was to investigate effects of polyphenol compounds derived from the shells of mangosteen, SSCM-034, on cAMP-activated intestinal Cl^- secretion. Short circuit current analysis of human intestinal (T84) cell monolayer showed that SSCM-034 inhibited both the cAMP and cholera toxin-activated Cl^- secretion in a dose-dependent manner with IC_{50} of ~100 mM. Analysis of Cl^- current across the apical membrane of T84 cells indicated that SSCM-034 targets two apical Cl^- channels activated by cAMP, cystic fibrosis transmembrane conductance regulator (CFTR) and unidentified inward rectifying Cl^- channels (IRC). This compound had no effect on cell viability as measured by MTT assays and did not alter intracellular cAMP levels in T84 cells. In addition, SSCM-034 blocked Ca^{2+}-activated Cl^- channels in this cell line with similar potency. Using apical Cl^- current measurements in Fisher rat thyroid (FRT) cells stably transfected with human CFTR, SSCM-034 inhibited CFTR-mediated apical Cl^- current activated by different CFTR agonists including cell permeable cAMP, IBMX (a phosphodiesterase inhibitor) and apigenin (a flavone-type direct CFTR activator), with equal IC_{50} of ~ 100 mM, indicating direct effects of this compound on CFTR. Finally, a single intraperitoneal injection of SSCM-034 (80 mg/kg) reduced cholera toxin-induced intestinal fluid secretion by 40% in mouse closed loop models. SSCM-034 represents a novel intestinal Cl^- channels blocker with potential utility in the treatment of diarrhea resulting from a broad spectrum of etiologies.

***Keywords**: CFTR, Diarrhea, Intestine, Mangosteen, Cl^- channel*

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Acetylcholinesterase inhibition potential and antioxidant activities of *Limnophila aromatica*

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Despite the increasing prevalence of Alzheimer's disease, the therapeutic efficacy of any treatment is still limited and debated; therefore, a novel protective strategy is required. Recent findings showed that substances possessing anti-cholinesterase and anti-oxidant activities could protect against this condition. Therefore, the present study was conducted to determine whether *Limnophila aromatic* a local indigenous vegetable belonging to the family Scrophulariaceae exhibits anti-cholinesterase and anti-oxidant activities. The aqueous extract of *L. aromatica* was tested for the anti-acetylcholinesterase activity by using the spectrophotometric method of Ellman using an ELISA microplate reader while the anti-oxidant activity was evaluated by determining the DPPH radical scavenging activity and ferric-reducing anti-oxidant power (FRAP). The total phenolic compound was also assessed using the Folin-Ciocalteu assay. The results showed that the extract of *L. aromatica*exhibited bothfree radical scavenger and anti-acetylcholinesterase activities. In addition, the extract contained atotal phenolic compound concentration of 340.81±3.53 mg/L Gallic acid equivalent. Our results suggest that *L aromatica*is a potential natural source for developing nootropic and neuroprotective agents against Alzheimer's disease albeit further *in vivo* research is essential.

Keywords: *Limnophila aromatic, anti-acetylcholinesterase, antioxidant, phenolic compound*

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Effect of *Anacardium occidentale* leaf extract on nerve function and oxidative stress in experimental diabetic neuropathy

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Diabetic neuropathy is one of the most common complications of diabetes mellitus. Hyperglycemia due to poor glycemic control can enhance oxidative stress resulting in diabetic neuropathy. Based on the longstanding reputation vis-à-vis the anti-diabetic and anti-oxidant activity of *Anacardium occidentale*, the current study was conducted to determine whether *A. occidentale* could improve the functional impairment of nerves affected by diabetic neuropathy. Male Wistar rats, weighing between 200 and 250 g, had a diabetic condition induced using streptozotocin. They then had neuropathy induced by inflicting a crush injury at the upper one-third of the right sciatic nerve. After the lesion induction, rats were treated with the alcoholic extract of *A. occidentale* leaves at doses of 25, 100 and 200 mg/kg BW via transdermal route for 21 days. The animals were assessed for functional recovery of the sciatic nerve by using the De Medinacelli Method and the foot withdrawal reflex test. At the end of experiment, the sciatic nerve was isolated and oxidative stress markers determined including (a) the level of malondialdehyde (MDA) and (b) the activities of scavenger enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The results indicate that all doses of *A. occidentale* leaf extract significantly improved the sciatic function index at day 15 of treatment but failed to show any significant change in the foot withdrawal reflex. Despite observing increased activity of CAT at all doses of treatment, no significant change of MDA was observed. Therefore, the ability of *A. occidentale* leaf extract to enhance functional recovery, as observed in this study, might not be associated with antioxidant activity. Understanding the precise underlying mechanism requires further investigation. Notwithstanding, this study suggests a potential role for *A. occidentale* leaf extract for facilitating functional recovery of diabetic neuropathy.

Keywords: *Anacardium occidentale, diabetic neuropathy, oxidative stress*

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Anti-thrombotic effects of rice bran peptide: a preliminary study

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Rice bran protein/peptide has reported anti-oxidant, anti-cancer and ACE inhibitory activities. We studied the anti-thrombotic activity of <5 kDa rice bran peptide. Anti-coagulant activity of Jasmin 105 rice bran peptide was assessed by incubating rice bran peptide at various concentrations (0, 0.025, 0.05, 0.1, 0.5, 1 and 2 mg/mL). Fibrinolytic activity was assessed and the fibrinolytic activity of euglobulin was monitored. No effects of rice bran peptide on PT were observed although there was a slight but significantly prolonged APTT clotting time seen at rice bran peptide concentrations of 0.5, 1 and 2 mg/mL. Increasing the incubation time of the peptide in plasma to 2 hr did not increase the anti-coagulant effect. When the peptide concentration was increased to 4, 8, 16 and 32 mg/mL, a striking anti-clotting activity on APTT over against PT was observed. At a peptide concentration of 32 mg/mL, prolongation of APTT clotting time was about 2.5-fold that of the baseline value, while heparin at a concentration of 0.25 i.u./mL demonstrated the APTT ~3 times that of the baseline value. As for fibrinolytic activity, no effects of rice bran peptide were seen on the AUC and time max slope at all studied concentrations. This is the first data to suggest an anti-coagulant property of rice bran peptide (MW <5 kDa). Further study is needed to explore its fibrinolytic activity.

Keywords: *rice bran peptide, antithrombotic effects, anticoagulant activity, fibrinolytic activity*

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Development of high anti-oxidative capacity and ready-to-eat soup products called "Aom Curry Soup"

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Oxidative stress has been found to be involved in the pathogenesis of many diseases. Therefore, convenient foods with a high anti-oxidative value would be useful for healthful living and disease prevention. This study aimed to develop nutritional instant dried soup products called "Aom Curry Soup". Freeze drying, spray drying and baking technologies were used to prepare the vegetables and spices. The anti-oxidative components and anti-oxidant activity were then determined using the HPLC technique and the DPPH scavenging assay, respectively. Three formulae for powdered Aom Curry Soup were developed including: pumpkin, tomato and carrot. The vitamin C, β-carotene, vitamin A and vitamin E contents of the three formulae ranged between 90.07-126.86, 319.21-514.54, 3.77-22.54 and 0.19-0.46 μg/g, respectively. Also, they showed relatively high antioxidant activity with EC_{50} values of 22.51-80.12 μg/mL. Aom Curry Soup products containing anti-oxidative vitamins with relatively high anti-oxidative activity were successfully developed from edible Thai vegetables and spices.

Keywords: *Aom Curry Soup, oxidative stress, anti-oxidant activity, ready-to-eat soup*

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Gait Event Detection Using Nintendo Wii

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Determination of gait events (*viz*., mid swing (MS), foot flat (FF), toe off (TO) and heel strike (HS)) is essential for clinical gait analysis, especially for patients with neuromuscular disorders. Combinations of devices (*e.g*., VICON and foot plates) are typically used to receive kinematic information. They are, however, usually expensive, time-consuming and cumbersome to use. In current study, the use of Nintendo Wii for detecting the gait events was investigated as a low-cost and easy-to-use alternative. Two Wii remotes with Wii Motion Plus were wrapped on both tibias of three healthy subjects. When the subjects walked on a flat surface, the devices would read 3-axis linear accelerations and 3-axis angular velocities. The MS event was determined from finding the local minima of the angular velocity. TO and HS events were then determined from mathematical equations of an average gait cycle period, calculated using autocorrelation of the angular velocity. The FF event was located at the middle of the consecutive TO and HS events, where we could verify that the sum of the 3-axis accelerations was 1*g* in magnitude. The HS events determined from both the angular velocity and the linear acceleration were in good agreement (coefficient of variation of the stride time was within 5% deviation). The study demonstrates that it is possible to use Nintendo Wii remotes for gait event detection.

Keywords: *gait event, mid swing, foot flat, toe off, heel strike, Nintendo Wii*

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Effect of Thai wand exercise on lung capacity among young sedentary adults

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Sedentary lifestyle and physical inactivity among young adults have been associated with cardiovascular disease (CVD). Poor cardiopulmonary fitness is associated with a high risk for all causes of mortality among sedentary individuals with or without underlying CVD. Thai wand exercise is a traditional exercise believed to improve health-related quality of life and reduce cardiovascular risk factors. The present study aimed to assess the effect of Thai wand exercise on lung capacity and cardiovascular risk factors among young sedentary adults. Twenty-nine young sedentary adults between 18 and 25 years of age were recruited. All of them performed Thai wand exercises 40 min/day, 3 days/week for 4 weeks. Anthropometry, body composition, blood pressure, chest expansion and lung capacity were measured before and after 4 weeks of training. Thai wand exercise significantly reduced waist circumference (71.5±5.7, 70.4±5.5 cm, $p<0.01$), percentage of body fat (35.4±4.0, 33.9±3.3, $p<0.01$) and fat mass (20.1±4.0, 19.2±3.6 kg, $p<0.01$) and increased tidal volume (0.5±0.2, 0.6±0.1 L, $p<0.01$), vital capacity (2.8±0.7, 2.9±0.7 L, $p<0.05$), forced vital capacity (3.0±0.6, 3.1±0.7 L, $p<0.01$) and chest expansion (upper; 3.2±1.0, 3.9±1.3 cm, $p<0.01$), (middle; 3.7±1.1, 4.4±1.2 cm, $p<0.01$), (lower; 3.8±1.3, 4.7±1.3 cm, $p<0.01$). The current study demonstrates that Thai wand exercises improve lung capacity which is related to an improved physical health status. In addition, this training reduced body and abdominal fat which are risk factors for CVD. Thai wand exercise could be a good approach for improving health-related quality of life and reducing cardiovascular risk factors.

Keywords: *Thai wand exercise, lung capacity, sedentary*

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Comparison between efficiency of exercise and anxiolytic drugs in the reduction of anxiety-like behaviors in stressed male rats

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Exercise has long been prescribed as an intervention in mitigating the symptoms of anxiety and mood disorders. The aims of this study were (*i*) to evaluate the anxiolytic effect of swimming (non-impact aerobic exercise) on the anxiety-like behaviors in chronic stressed rats, and (*ii*) to compare the efficiency between anxiolytic drug treatments and swimming. Male Wistar rats exposed to restraint stress (1 h/day, 5 days/week) were subjected for 4 weeks to exercise training (swimming 1 h/day, 5 days/week), various anxiolytic drug treatments (i.e., 2 mg/kg diazepam; 10 mg/kg fluoxetine; 10 mg/kg reboxetine; or 10 mg/kg venlafaxine p.o.), or the combined swimming and anxiolytic drug treatment. All rats were weekly subjected to the sucrose intake test for evaluation of stress. At the end of the 4-week drug treatment and/or swimming, anxiety-like behaviors were determined by the elevated plus-maze (EPM), elevated T-maze (ETM) and open-field tests. The results showed that swimming rats had a reduction in body weight gain with cardiac hypertrophy when compared to sedentary rats. An increase in the open arm time in the EPM test was observed in the swimming, reboxetine-treated, and venlafaxine-treated groups, while a decrease in avoidance latency from the baseline to avoidance-1 and avoidance-2 in the ETM test was observed in the swimming and venlafaxine-treated groups. Only venlafaxine showed a synergistic anxiolytic-like action with the 4-week swimming. However, the amount of sucrose consumption and the total lines crossed in the open-field test in all groups were not altered, suggesting that neither swimming nor anxiolytic drugs affected stress and general locomotor activity, respectively. In conclusion, swimming could help to reduce anxiety-like behaviors in stressed male rats with equivalent potency to typical anxiolytic drugs. The combined swimming and venlafaxine treatment may further benefit stressed individuals with anxiety.

Keywords: *anxiety, elevated-plus maze, elevated-T maze, open-field test, swimming*

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Asporin expression in mouse alveogenesis

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The development of functional alveoli (or alveogenesis) contributes to an increase in the areas of gas exchange. Several chronic lung diseases are associated with an impairment of alveolar development and repair. It has been reported that the lung pathology of mice harboring the mutated fibroblast growth factor receptor type 3 and 4 (FGFR3/4 compound knockout) is observed in bronchopulmonary dysplasia and pulmonary emphysema. Interestingly, gene expression of aspirin an extracellular matrix protein is significantly elevated in the lungs of FGFR3/4 compound knockout mice, as detected by microarray study. Currently, the biology of asporin in the lung has been minimally defined. We hypothesized that asporin expression in the lung is associated with alveogenesis; thus, the current study aimed to identify the temporal and spatial expression of asporin in normal mouse lung during alveogenesis. We used real time PCR and the immunofluorescence technique to identify the pattern of asporin expression in mouse lungs. We identified the peak of asporin expression in mouse lungs was observed on postnatal day (P) 7-14, as related to the alveolar stage of mouse lung development, similar to the FGFR3/4 expression pattern. Asporin gene expression in FGFR3/4 compound knockout mice was highly elevated during P7- P28, compared with age matched, wild type mice. Asporin immunolocalization in adult mouse lungs (P60) was shown in the area corresponding to smooth muscle cells. In conclusion, asporin expression in the lung might be regulated by FGFR3/4 signaling and therefore affect alveogenesis

Keywords*: asporin, alveogenesis*

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Protective effect of testosterone on death of pancreatic β-cells cultured in high-glucose

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Type 2 diabetes (T2D) is a metabolic disease characterized by chronic hyperglycemia resulting from failure of pancreatic β-cells to secrete sufficient insulin to compensate for insulin resistance of body tissues. Hyperglycemia can in turn induce pancreatic β-cell death through the production of reactive oxygen species. The increased incidence of T2D correlates with aging. Epidemiological studies indicate that the increment of T2D in males is associated with a low testosterone level. Testosterone is the male steroid hormone which has an anti-oxidant effect. A recent study showed that testosterone replacement protected pancreatic β-cell apoptosis in streptozotocin (STZ)-induced diabetic rats through the induction of anti-oxidant enzymes. Thus, it is possible that testosterone can prevent β-cell death induced by hyperglycemia. This research aimed to study the effect of testosterone on β- (INS-1) cell death after culturing in a medium containing a high level of glucose. Testosterone at final concentrations of 0.05, 0.5, 1 and 2 μg/ml, respectively, was added to cultures of β-cells in media containing normal glucose (11.1 mM) or high glucose (40 mM) for 72 hours. After staining with propidium iodide (PI), the respective number of dead β-cells was counted. When cultured in high-glucose medium, the respective number of dead β-cells was significantly higher than when cultured in normal-glucose medium. While testosterone at all concentrations did not show any effect on the death of β-cells cultured in normal-glucose medium, testosterone at 0.05 and 0.5μg/ml significantly reduced the respective number of dead β-cells cultured in high-glucose medium. The protective effect of testosterone was, however, diminished when its concentrations were high (1 and 2 μg/ml). The mechanism of how testosterone prevents β-cell death in high-glucose medium is being investigated.

Keywords: *type 2 diabetes, T2D, hyperglycemia, testosterone, β-cell death*

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Effect of estrogen on INS-1 pancreatic β-cell apoptosis cultured in high glucose

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The prevalence of diabetes mellitus will soon number 246 million people worldwide. Type 2 diabetes is a metabolic disorder comprising insulin resistance and β-cell dysfunction leading to hyperglycemia. Chronic hyperglycemia results in β-cell apoptosis through oxidative stress and ER stress. Estrogen is a female sex hormone which exhibits anti-inflammatory and anti-oxidant effects. In our previous studies, estrogen was found to improve glucose-stimulated insulin secretion in mouse pancreatic islets cultured in high glucose medium for 10 days. Estrogen also increased the anti-apoptotic *bcl-2* mRNA level in human breast cancer cells. It is hypothesized that estrogen decreases pancreatic β-cell apoptosis resulting from prolonged culture under conditions of high glucose concentrations. Our objective was to determine whether 17β-estradiol would decrease pancreatic β-cell apoptosis after prolonged culturing in a high glucose medium. Annexin V / Propidium Iodide (PI) staining was performed on INS-1 pancreatic β-cells incubated with 11.1 mM glucose (control) or 40 mM glucose (high glucose) RPMI 1640 medium with or without 10^{-8} M 17β-estradiol for 72 hours. The INS-1 cells were trysinized and re-suspended in media. The INS-1 cells were incubated with Annexin V FITC for 15 minutes then PI was added to the samples. The samples were analyzed immediately by flow cytometry. Early apoptosis was found increased in the cells cultured in high concentrations of glucose compared to the control. The apoptotic cell number of INS-1 cells cultured in high glucose medium with 10^{-8} M 17β-estradiol was reduced compared to the same condition without 10^{-8} M 17β-estradiol. Our study demonstrated that 17β-estradiol decreased pancreatic β-cell apoptosis after culturing in media high in glucose.

Keywords*: estrogen, INS-1 cells, high glucose, apoptosis*

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Effect of estrogen on angiotensin II type 1 receptor expression in pancreatic β-cell prolonged culture in high glucose

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The risk of developing type 2 diabetes increases after menopause but it is reported that estrogen replacement therapy can decrease the risk. Local pancreatic islet angiotensin II type 1 receptor (AT1R) is up-regulated during hyperglycemia leading to the impairment of insulin secretion. Estrogen has a role in AT1R expression in various tissues such as the adrenal cortex and pituitary gland. The role of estrogen on AT1R expression in pancreatic β-cells with hyperglycemia remains unclear. This study aimed to investigate the effect of estrogen on AT1R expression in pancreatic β-cells after prolonged culturing in a high glucose medium.

INS-1pancreatic β-cells were cultured in medium containing a normal glucose concentration (11.1mM) and a high glucose concentration (40 mM), with and without 10^{-8} M 17β-estradiol. After 48 hrs, total RNA was extracted and reverse transcribed to cDNA. Real time polymerase chain reaction was performed to demonstrate AT1R gene expression. The expression of AT1R gene is significantly increased in cells cultured in a high glucose medium compared to a normal glucose medium. Moreover, it was found that estrogen significantly decreased high glucose-stimulated AT1R gene expression. Prolonged culture of INS-1 cells in a high glucose medium can up-regulate AT1R expression, while 17β-estradiol can restore AT1R expression. These findings demonstrate the inhibitory effect of estrogen on high glucose-induced AT1R gene expression in pancreatic β-cells exposed to a high glucose medium for a prolonged period.

Keywords*: estrogen, Ins-1 cells, high glucose, angiotensin II type1 receptor*

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Passive immunization against inhibin-alpha subunit in weaning female rats induced early onset of puberty and sexual maturation

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Previous studies have shown that inhibin-alpha antiserum induced superovulation in both mature and immature female rats, but the effect on puberty and sexual maturation has not been reported. This study aims to determine the onset of puberty and sexual development of weaning female rats after passive immunization against inhibin-alpha subunit. Weaning Wistar female rats received inhibin antiserum (1 μg/100 μl, i.p.) or saline (100 μl, i.p., control) on Day 26, 27, 28, 29, and 34 postnatal. Vaginal opening was monitored on Day 26 through Day 41. Then, vaginal smears were performed for 4 consecutive days. On Day 45, all rats were sacrificed and reproductive organs were removed, weighed, and fixed for histological examinations. Blood samples were collected from the abdominal vein for hormone assay using ELISA. Passive immunization had no effect on the body weight over the period of treatment, but induced an early opening of vagina (on Day 35 compared to Day 41 in control rats). By Day 45, immunized rats had significantly ($p<0.01$) higher ovarian and uterine weights compared to controls. Ovarian histology showed increases ($p<0.01$) in number and size of the follicles, which are predominantly primary and Graffian follicles. Uterine of the immunized rats exhibited proliferative endometrium with uterine glands compared to the undifferentiated uterine layers in the controls. Serum FSH, LH and estradiol of the immunized rats were significantly higher than controls whereas serum progesterone showed only a trend to increase, but not significant. The results suggest that immnunoneutralization of the endogenous inhibin enhances pituitary secretion of FSH, which stimulates the development and growth of the follicles. The latter, in turn, secrete estradiol required for sexual maturation. It is concluded that passive immunization against inhibin-alpha during postnatal age accelerates the onset of puberty and sexual maturation.

Keywords: *passive immunization, inhibin-alpha, puberty, sexual maturation, female rats*

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Comparative study on post-washed semen parameters after semen preparation by discontinuous density gradient centrifugation technique or swim up method

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Ten to 15% of couples seek medical help due to infertility. Poor semen quality accounts for ~20% of infertile couples. Several semen preparation techniques have been developed to maximize the chance of infertility treatment. We compared the post-washed semen parameters of semen samples after being processed using a density gradient centrifugation technique (or swim up method). Semen samples were collected from 35 male subjects. After liquefaction, small amount of each fresh semen sample was analyzed for sperm count and motility rate using Computer Assisted Semen Analysis (CASA). A total of 1 ml of each sample was aspirated into 2 aliquots to process by two different preparation techniques; namely, the swim up method and the density gradient centrifugation method. The semen samples were then further analyzed for sperm count, motility rate and apoptosis rate. The percentage of progressive motile sperm was significantly higher in the samples prepared by the density gradient centrifugation technique vs. the swim up method (83.7$\pm$6.9% vs. 66.09$\pm$4.5%, P<0.05). The total motile sperm count, curvilinear velocity (VCL) and average path velocity (VAP) of samples prepared by density gradient centrifugation technique were significantly better than those processed by swim up method. Flow cytometry demonstrated that the total apoptotic rate was significantly higher in the samples processed using the swim up technique vs. the density gradient centrifugation method (13.99$\pm$6.10% vs. 9.42$\pm$3.45%, P < 0.001). This study demonstrated that the density gradient centrifugation technique yielded better quality semen samples than the swim up method.

Keywords: *sperm motility, sperm apoptosis, swim up method, density gradient centrifugation method*

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Success rate of intrauterine tuboperitoneal insemination at Srinagarind Hospital

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More than one in six couples seeks medical advice because of infertility. Non-tubal infertility accounts for between 50 and 70% of infertile couples. Several treatment modalities have been developed to maximize the chance of conception in these groups of patients but intrauterine insemination (IUI) has been the mainstay of therapy for couples suffering from non-tubal infertility. The success rate of IUI has, however, been rather low. Some new alternatives such as intrauterine tuboperitoneal insemination (IUTPI) have therefore been proposed. The objective of this study was to determine the success rate of intrauterine tuboperitoneal insemination (IUTPI) as performed at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. In this study, the subjects included sixteen couples with non-tubal infertility who met the inclusion criteria (unexplained infertility, minimal-mild endometriosis, mild oligozoospermia). After ovarian stimulation with clomiphene citrate all of the subjects underwent intrauterine tuboperitoneal insemination (IUTPI) at between 36 and 40 hr after administration of hCG. All 16 treatment cycles were included in this study. The clinical pregnancy rate per cycle was 6.3% (1/16). No multiple pregnancy, ectopic pregnancy, ovarian hyperstimulation syndrome or pelvic infection was detected in this study. This study suggests that IUTPI, after ovarian stimulation with clomiphene citrate, provided a reasonable success rate in patients suffering from non-tubal infertility.

Keywords: *intrauterine tuboperitoneal insemination, pregnancy rate*

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Effectiveness of structural modification program with primary dysmenorrhea in adolescent students

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Primary dysmenorrhea is one of the most common gynecologic problems among adolescents. To cope, adolescents use various strategies including medical and non-medical approaches. This current study compared pain levels before and after organized daily adjustment to posture by using a structural modification program. The sample population used in the study had primary dysmenorrhea and a perceived level of pain during the menstrual period ≥ 4. This study used a quasi-equivalent control group design, in which the pain level was compared before and after attending the structural modification program between May and July, 2009. The tools used in this study comprised: Part I the personal data (13 items); Part 2 assessed pain scores from 0-10; and, Part 3 a form to record morning and evening performance of posture modification. The results support the hypothesis that the structural modification program could relieve pain during the menstrual period ($p<0.05$).

Keywords: *structural modification, primary dysmenorrheal, adolescent*

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Protective effects of exercise on depression-induced bone change in ovariectomized rats

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Depression in menopausal woman is characterized by changes in mood such as sadness, feeling of hopelessness, less motivation and less physical activity. Interestingly, it is also associated with low bone mineral density (BMD;g/cm^2) and content (BMC;gram) and increased incidence of osteoporotic fractures. Estrogen (E_2) has been known to have therapeutic effects, i.e. anti-depression and prevention of osteoporosis. Physical exercise, which can be classified into impact/non-impact physical training and forced/voluntary exercise, also has similar beneficial effects. However, it is not known whether a combination of exercise and estrogen supplementation would have combined preventive effects against bone loss in depression. The purpose of this study was to investigate the antidepressant like effects of estrogen combined with different types of exercise training on bone of ovariectomized (OVX) rats with chronic mild stress (CMS). Bilaterally OVX 8 week-old female Wistar rats, that had been exposed to 4 week-CMS protocol, were used as a depressed menopausal osteoporosis animal model. Rats were assigned into 8 groups i.e. OVX with/without CMS, OVX+CMS and 4 week-swimming (non-impact exercise) or voluntary wheel running (impact exercise) and OVX+CMS+10 mg/kg of estrogen supplement (E_2) with and without exercises. After training, animals were sacrificed and tibias were used for determination of BMC and BMD by DEXA. Results showed that, as expected, E_2 significantly increased tibia BMD of OVX rats. In contrast, CMS reduced the BMC of OVX rats. Both impact and non-impact exercises were found to prevent the negative effect of CMS on BMC and BMD. Moreover, both types of exercise could increase BMC but not BMD in OVX+CMS rats receiving E_2 supplement. In conclusion, these results reveal the efficiency of both impact and non-impact exercises preventing bone mineral loss in depressed ovariectomized rats. In addition to the positive effect of E_2 on bone, exercise further increased the tibia BMC of these animals.

Keywords: *swimming, voluntary running, estrogen, bone density, chronic mild stress*

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Effect of tetramethylpyrazine on ionic currents in human coronary artery endothelial cells

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Tetramethylpyrazine (TMP) was reported to cause vasodilation and reduce arterial pressure. In vascular smooth muscle cells (VSMCs), TMP induced hyperpolarization by affecting VSMC ionic channels. Additionally, TMP could elicit endothelium-dependent pulmonary artery relaxation. Therefore, we hypothesized that TMP may similarly affect ionic currents of human coronary artery endothelial cells (HCAECs). Cultured HCAECs were studied using whole-cell patch clamp technique. Cells were stimulated with 600-ms pulses from -40 mV holding potential to -100, -80, -60 … +80 mV. TMP at 3 and 30 μM significantly decreased current densities at +60mV to 73.01 ± 10.56 % (n=7) and 78.65 ± 5.81 % (n=7), respectively. To test the TMP effects on small and intermediate conductance Ca^{2+}-sensitive K^+ channels (SK_{Ca} and IK_{Ca}), inward rectifier K^+ channels (K_{ir}) and TRP channels, specific blockers were used (100 nM apamin, 10 μM clotrimazole, 100 μM Ba^{2+} and 10 μM La^{3+}, respectively) When most channels were blocked, leaving primarily SK_{Ca} or IK_{Ca} currents, 30 μM TMP decreased the current density to 61.04 ± 20.00 % (n=3) and 50.25 ± 5.08 % (n=3). TMP did not affect currents through other channels in similar experiments. These data suggested that TMP could diminish HCAEC currents, and this effect could be mediated by SK_{Ca} and/or IK_{Ca} inhibition. Further investigation will elucidate the signaling pathways involved.
Keywords: *tetramethylpyrazine, K^+ channel, human coronary artery endothelial cell*

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Effect of PPAR gene polymorphisms on cardiovascular disease risk in Thais

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Peroxisome proliferator activated receptors (PPARs) are nuclear hormone receptors, which play important roles in metabolic disorders and vascular inflammation related to atherosclerosis. Both the *PPARα* and *PPARγ* gene polymorphisms have been implicated in the development of metabolic syndrome, type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). The objective of the present study was to evaluate the association between *PPARs* polymorphisms and risk factors of coronary atherosclerosis in Thais. Polymorphisms of *PPARα* (*V227A)* and *PPARγ (P12A)* were genotyped in subjects with dyslipidemia and a control group, using an allele-specific polymerase chain reaction (AS-PCR) technique developed *in house*. The genotypes were evaluated for their association with lipid profiles, fasting blood glucose and diabetic condition of the patient. The frequencies of the minor alleles, *PPARα227A* and *PPARγ12A*, in the studied population were 0.023 and 0.024, respectively. Among individuals with dyslipidemia, carriers of *227A* were associated with lower total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and non-high density lipoprotein cholesterol (non-HDL-C), as compared with those of *227V* ($P<0.05$). By contrast, the *12A* carriers of *PPARgγ P12A* were associated with a higher level of TG and TG/HDL than those subjects with the *12P* allele ($P<0.05$). The percentage of *12A* in the diabetic group (2.13%) was lower than in the non-diabetic group (6.015%). Moreover, carriers of the *12A* allele exhibited a lower level of fasting blood sugar than those with *12P*. The frequency of the *PPAR* polymorphisms, *V227A* and *P12A*, are low in Thais. The occurrence of *227A* may, however, be associated with a lower risk of atherogenic lipid levels, and the *12A* allele with a lower risk of developing diabetes.

Keywords: *PPARs, polymorphisms, coronary artery disease (CAD)*

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Antioxidant and anti-allergy activities of Thai traditional medicine preparations

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A Thai traditional medicine preparation called *Harak*, comprises five roots including, *Ficus racemosa* Linn., *Capparis micracantha* DC., *Clerodendrum indicum* (Linn) O. Ktze, *Harrisonia perforata* Merr., *Tiliacora triandra* Diels. It is used as an anti-allergy preparation in Thai traditional medicine. Thus, the objectives of this research were to investigate (a) the antioxidant effect using the DPPH assay and (b) the anti-allergy potential by examining its inhibitory activity against b-hexosaminidase release in RBL-2H3 cells of the *Harak* and its constituents. This preparation and each plant component were macerated with 95% ethanol then dried in an evaporator. *Harak* demonstrated a level of high anti-allergy activity at IC_{50} values < 20 μg/ml but less antioxidant activity at EC_{50} values of 40.93±1.25 μg/mL. The plant in this preparation showing the highest anti-allergy activity was *C. micracantha* (IC50 value of 9.80 μg/mL). *F. racemosa* showed the highest antioxidant activity followed by *T. triandra* and *H. perforate* (EC_{50} = 4.87±0.24, 15.38±0.25 and 16.91±0.73 μg/mL, respectively). These results support the use of this preparation as an anti-allergy drug as done in Thai traditional medicine; however, further research should be done to isolate the active compounds.

Keywords: *Ha-Rak, antioxidant, anti-allergy, β-hexosaminidase release, RBL-2H3*

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Antimicrobial and Antioxidant Activities of Thai medicinal plants for AIDs patients

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Opportunistic infections in AIDs patients is the leading cause of death in AIDs patients. HIV infection reportedly causes an increase in oxidative stress which may in turn lead to faster progression of the syndrome. Thus, medicinal plants with demonstrated antimicrobial and antioxidant activities would be of therapeutic value for AIDs patients. Our objectives were (a) to investigate the antimicrobial and antioxidant activity of three Thai medicinal plants selected by Thai folk doctors for treatment of AIDs patients including, *Dioscorea bulbifera* (DB), *Momordica charantia* (MC) and *Carica papaya* (CP). Ethanolic and water extraction methods were both tested. Antioxidant activity was measured using the DPPH assay and antimicrobial activity using the disc diffusion assay and minimal inhibitory concentration (MICs) assay against *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*. The total phenolic contents were also tested using the Folin-Ciocalteau colorimetric method. The ethanolic extract of DB showed the highest antioxidant activity (EC_{50} values as 11.07 μg/mL) while the other extracts had no antioxidant activity (EC_{50} >50 μg/mlL). The same extract showed the most active antimicrobial activity against both gram positive and negative bacteria (*viz., S. aureus*, *B. subtilis*, *E. coli*. The ethanolic extract of MC and CP was active only against *B. subtilis*. The water extract of the three plants had no antimicrobial activity. The total phenolic content of these extracts correlated with DPPH radical scavenging activity**.** We conclude that *D. bulbifera,* used as an ingredient in a traditional Thai medicine HIV preparation, should undergo further study for its inhibition of enzyme HIV-1 integrase, protease and transcriptase.

Keywords: *Dioscorea bulbifera, Momordica charantia, Carica papaya, Antimicrobial activity, antioxidant activity*

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Anti-oxidant, anti-bacterial and cytotoxic activities against human cells of *Tectona grandis*

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Tectona grandis is popularly used in Thai medicinal preparations for cancer treatment. The objectives of this research were to investigate the anti-oxidant, anti-microbial and cytotoxic activities against human cancer cells. Wood of *T. grandis* was macerated by 95% ethanol and dried by an evaporator. The cytotoxic activity was tested against two types of human cancer cells (*i.e.,* A549, CORL-23) in one type each of breast and prostate cancer cells (*viz.,* MCF-7 and PC3) and determined by SRB assay. The anti-bacterial activity against two types of gram positive bacteria (*Staphylococus aureus* and *Bacillus subtilis*), one type of gram negative bacteria (*Escherichia coli*) and one type of yeast (*Candida albicans)* were evaluated by disc diffusion method. The active extracts were diluted to determine the minimum inhibitory concentration (MIC) by agar dilution method. We found that the ethanolic extract of *T. grandis* showed good cytotoxic activity against all cancer cells at IC_{50} values < 30 μg/mL for each cell line. The extract also showed good anti-oxidant activity by EC_{50} values as 25 μg/mL. It also showed anti-bacterial activity against both gram positive bacteria *S. aureus,* and *B. subtilis* (MIC = 0.3125 and 0.625 mg/mL, respectively). These results provide evidential support for the use of this plant in Thai traditional medicine to treat cancer patients and chronic wound infection. Further research is needed on isolating the cytotoxic and anti-microbial agents from this plant extract for cancer treatment.

Keywords: *Tectona grandis, cytotoxic activity, antimicrobial activity, antioxidant activity*

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Expression of recombinant aquaporin 4 protein for detection of anti-aquaporin 4 in neuromyelitis optica disease

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Neuromyelitis optica (NMO) is an autoimmune inflammatory disease of the central nervous system, which the optic nerves and spinal cord causing optic neuritis with longitudinally extensive transverse myelitis. These symptoms are similar to other demyelinating disease, particularly multiple sclerosis (MS). Being able to discriminate between NMO and MS is necessary because the treatment regimens are different. There is a unique bio-marker in NMO patients called NMO IgG. This antibody recognizes Aquaporin 4 a water channel expressed on the surface of astrocytes in the brain and the detection of which has been widely used for early diagnosis of NMO disease via indirect immufluorescent, using either mouse brains or AQP4-transfected cells. This technique has both high sensitivity and specificity but is expensive. In this study, soluble AQP4-GFP fusion protein in *E. coli*was used for detection of NMO IgG in serum from patients using an enzyme-linked immunosorbent assay (ELISA). With a serum dilution of 1:9, this test yielded high sensitivity (97.5%) and specificity (100%) comparable to other methods (n=6).

Keywords: *neuromyelitis optica (NMO), aquaporin 4 (AQP4)*

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Effect of aromatherapy massage on perception of pain during labor

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Researchers hypothesized that aromatherapy massage (AM) could help alleviate labor pain. This quasi-experimental research aimed (a) to compare perceived pain during labor between the parturient receiving pregnancy care with AM and the usual care and (b) to compare satisfaction with the childbirth experience during the active phase of labor between parturients receiving AM and those receiving usual care. There were two groups of 39 participants. Twenty participants were assigned to the experimental group received back, shoulder and neck massage with lavender oil; another 19, assigned to the control group, received the same massage but without the oil. Data collection tools included VAS, questionnaire on general information and on satisfaction with the childbirth experience. The AM included a CD describing the program and lavender massage oil. The data were analyzed for frequency, percentage, mean and independent t-test. The perception of pain score during the active phase of labor at 10 minutes after intervention was not different between the experimental and control groups ($p < 0.05$). The average satisfaction score of the childbirth experience in the experimental group was higher than the control group ($p < 0.05$). These results do not support the use of aromatherapy massage with lavender oil to relieve labor pain, but the therapy does significantly increase the satisfaction of the parturient with the labor experience. The program of aromatherapy massage in labor pain period should be considered for parturients during the active phase of labor.

Keywords: *aromatherapy massage, labor pain*

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Identification of chalcone as an activator of CFTR chloride channels

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel expressed in epithelia of various organs such as lung pancreas and intestine. It is primarily located on the apical site of the cell membrane where it plays a key role in controlling fluid and electrolyte transport. Loss of functions of CFTR leads to cystic fibrosis (CF), a lethal genetic disease in Caucasians. The low activity of CFTR resulted in an excessively viscous mucous and impaired mucociliary clearance, making CF patients more vulnerable to recurrent infections with bacteria, which eventually end up with respiratory failure. Activators of CFTR therefore represent a promising therapeutic approach for cystic fibrosis. Recently, it was found that flavonoids, a plant - derived compounds, stimulate CFTR activity in Calu-3 cells. In this study we aimed to investigate the effect of chalcone, intermediates of flavonoid biosynthesis, in modulating CFTR activity in a heterologous expression system of human CFTR, the Fisher rat thyroid cells stably transfected with human CFTR. Apical chloride current analysis showed that a representative chalcone, ASCM-007, stimulated CFTR-mediated chloride current primed by low dose of forskolin, an adenylate cyclase activator. Maximum response was obtained at 100 μM of ASCM-007. Due to the fact that the function of CFTR is regulated by intracellular cAMP levels, effects of ASCM-007 on intracellular cAMP levels were investigated by an immunological assay of cAMP. We found that ASCM-007 did not have an effect on intracellular cAMP levels. In addition, cell viability assay showed that ASCM-007 at concentrations of 10-100 μM had no cytotoxicity to FRT cells. In conclusion, the present study identified chalcone as a novel chemical class of CFTR activators.

Keywords: *CFTR, CFTR activator, natural products, cystic fibrosis, flavonoid*

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Effects of *Scaphium scaphigerum* (G.Don) Guib & Planoh on abdominal adipose tissue, weight loss and leptin hormone in Thai obesity

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Obesity and weight-related health problems have reached alarming rates in Thailand. The Thai government has spent many millions of baht for treatments of obese patients. Thai herbs, Malva nuts and *Scaphium scaphigerum* (G.Don) Guib & Planoh contain high water-soluble dietary fiber. They are consumed in Thai and Chinese cuisine to decrease the occurrence of obesity by acting as a bulking agent. The aim of this study is to evaluate the effects of *Scaphium scaphigerum* (G.Don) Guib & Planoh on abdominal adipose tissue, weight loss and leptin hormone in Thai obesity. A total of 28 healthy obese Thai women, aged between 35-60 years, were divided into two groups. The first group is classified as obese I followed by WHO criteria (BMI≥30-34.99 kg/m^2) and the second group is obese II (BMI≥35kg/m^2). Each group was divided into two subgroups; the control and the treatment group. The control group received one bottle of brown jelly and the treatment group received Malva nuts supplement containing 0.08% of body weight for 8 weeks. The results showed Malva nuts significantly decreased body weight, BMI, body fat percentage, cholesterol and triglyceride for the obese I group. While Malva nuts only significantly decreased body fat percentage for the obese II group. The data of respiratory exchange ratio (RER) and serum leptin hormone has shown no significance in both obese I and obese II. Thus the *Scaphium scaphigerum* (G.Don) Guib & Planoh has shown decrease effects only in weight loss parameters in Thai obesity.

Keywords: *obesity, Malva nuts, leptin hormone, abdominal adipose tissue*

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Steviol, a derivative of natural sweentener stevioside, slow cyst enlargement in an in vitro model of polycystic kidney disease (PKD)

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Increased levels of intracellular cAMP play an important role in PKD cyst growth by stimulating proliferation of cyst-lining cells and heightening transepithelial fluid secretion into the cyst lumen. A previous study found that steviol inhibits cAMP-activated chloride secretion by targeting CFTR in human colonic epithelial cells. We therefore studied the effect of steviol and its derivatives on cyst growth and cyst formation using an in vitro model of PKD. Type I Madin-Darby canine kidney (MDCK) cells were used to generate cysts in three-dimensional collagen gel in the presence of forskolin. Among 4 steviol-related compounds, steviol was found the most potent derivative retarding cyst growth in a dose-dependent manner. Steviol at 100 μM significantly inhibited cyst growth and cyst formation by 38.3% and 72.4 %, respectively. The effects of steviol in inhibiting cyst growth were reversible and MTT assays showed that steviol at doses up to 200 μM has no cytotoxic effects. We examined the underlying mechanism putatively responsible for the effects of steviol on cyst enlargement. The apical chloride current of MDCK cell monolayers was measured using Ussing chamber experiments after basolateral membrane permeabilization and steviol partially inhibited forskolin-stimulated apical chloride current, suggesting that steviol retards cyst progression, at least in part, by inhibiting apical CFTR chloride channels. Our data indicated that steviol retarded cyst growth in an in vitro PKD cyst model. It, therefore, represents a candidate drug for polycystic kidney disease and further study is needed to define the detailed mechanism of action.

Keywords: *steviol, CFTR chloride channel, cyst growth, polycystic kidney disease*

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Multipex allele-specific polymerase chain reaction for determination of plasminogen activator inhibitor type-1 (PAI-1) 4G/5G polymorphism in dyslipidemia

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Increased activity of plasminogen activator inhibitor type-1 (PAI-1) has been shown to be associated with an increased risk of thrombotic events in coronary artery disease. Moreover, a relationship between PAI-1 activity with triglyceride has also been identified. Since 4G allele of PAI-1 4G/5G polymorphism has been associated with higher PAI-1 activity, we investigated the association(s) of polymorphism and dyslipidemia by using multiplex allele specific polymerase chain reaction. A total of 223 subjects attending the Medical Laboratory and Physical Therapy Clinic, Faculty of Associated Medical Sciences, Khon Kaen University, for a health check were recruited to the study. According to the lipid profile results, the subjects were classified as dyslipidemic (n=102) and normolipidemic (n=121). PAI-1 4G/5G polymorphism was determined in all of the subjects using multiplex allele specific polymerase chain reaction. Four oligonucleotide primers were designed by primer designing tools, as follows: (1) control forward primer 5'CTTACACGTTGGTCTCC TGTTTCC3' (2) control reverse primer 5'AGCCAGCCACGTGATTGTCTAG3' (3) forward primer for 4G allele 5'AGAGTCTGGACACGTGGTGA3' and (4) reverse primer for 5G allele 5'GATGATACACGGCTGACTCCTCC3'. All 4 primers were applied in a single tube reaction. There were no significant differences in the genotype frequencies of the polymorphism between the dyslipidemic and normolipidemic groups (4G/4G=28.4 vs. 21.5%, 4G/5G = 51.0 vs. 57.9% and 5G/5G = 19.6 vs. 18.2%, p=0.51). The association between PAI-1 4G/5G polymorphism and dyslipidemias was not demonstrated in this study.

Keywords: *plasminogen activator inhibitor type-1 (PAI-1), polymorphism*

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Azide induced astrocyte death as an *in vitro* chemical hypoxic/ischemic model

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Azide is an effective and specific inhibitor of cytochrome oxidase complex IV that is a rate-limiting enzyme in oxidative phosphorylation and exposure induces cell death. Since limited information is available concerning the relative effect of chemical ischemia-induced cell death on astrocytes, we investigated the time- and dose-dependent toxicity of azide on primary culture astrocytes. Astrocytes were seeded at a density of $5x10^4$ cells/mL. Sodium azide was added at varying concentrations from 1 to 10 mM in a glucose-free medium for 30 min. Cell death and total cell number were quantified using propidium iodide and Hoechst 33258 staining, respectively. Azide induced a dose-dependent increase in cell death. Some astrocyte death was observed upon exposure of astrocytes to 1-2.5 mM azide, but this was not statistically different from the control. The astrocyte death was significantly increased to 19% at 5 mM azide (19.42±0.89% compared to the control, 0.24±0.14%) and 40-45% at the highest concentration of azide (40.27±0.85% for 7.5mM and 45.96±2.50% for 10mM). Astrocyte cultures were also exposed to 10 mM azide in a glucose-free medium for 5-60 min. The percentage of astrocyte death after 5-15 min-exposure of azide was not significantly different from the control, however, increasing the duration of treatment to 30 and 60 min resulted in a significant increase in cell death (*viz*., 45.96±2.50% of the control and 84.41±12.89%, respectively). Azide induced astrocyte death in a concentration- and time-dependent manner; the optimal concentration and duration of azide incubation might therefore considered as a protocol for screening potential protective agents against astrocyte death.

***Keywords**: astrocyte, sodium azide, chemical ischemia, cell death*

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Anti-proliferative activities of pure compounds from *Diospyros* filipendula and *Diospyros* cauliflora

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Many *Diospyros* species have been reported in traditional medicine. They exhibit interesting pharmacological activities. Almost all parts of these plants have a great diversity of compounds ranging from hydrocarbons, steroids, terpenoids and naphthoquinones. Many species of *Diospyros* contain compounds with cytotoxic properties and induce apoptosis but *D*. filipendula and *D*. cauliflora are still lack of medicinal record in the current literature. The objective of this study was to evaluate antiproliferative activities including molecular mechanisms in human cervical cancer cells. Crude hexane extracts of roots of *D. filipendula* and *D. cauliflora* were purified by chromatography and crystallization. Stigmasterol and taraxerol from *D. filipendula* and lupeol from *D. cauliflor*a have been isolated. Using HeLa cells as a model system, the cytotoxic effects were measured by a MTT assay. Following various treatments, DNA samples were electrophoresed on a 1.5% agarose gel and apoptotic nuclei were quantified using DAPI and propidium iodide (PI) staining. Stigmasterol, taraxerol and lupeol induced HeLa cell death in a dose-dependent manner associated with rounding cells, membrane blebbing and apoptotic body compared with polygonal shape in control cells. The IC_{50} of 48 h incubation was 37 ± 2.49, 10 ± 1.3 and 20 ± 2.6 μg/ml, respectively. DNA agarose gel electrophoresis showed typical length of DNA fragmentation where as control cells did not provide smear bands. The apoptotic nuclei of treated cells were 30.27 ± 0.9, 23.8 ± 0.99 and 42.23 ± 2.51% respectively compared with the untreated control (3.12 ± 1.29%). Results suggested that stigmasterol and taraxerol from *D. filipendula* and lupeol from *D. cauliflor*a had cytotoxicity and induction of apoptosis on HeLa cells. These substances represent dietary phytochemicals which may show different activities and have potential for cancer chemoprevention. To gain insight into mechanisms of apoptosis, the role of caspase activation cascade needs to be explored.

Keywords: *Diospyros filipendula, Diospyros cauliflora, HeLa cells, anti-proliferation, apoptosis, DNA fragmentation*

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Wii-based for knee joint angle measurement

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Biomechanical information is increasingly important for rehabilitation purposes. The inertial measurement unit (IMU), which is a combination of gyroscopes and accelerometers that are capable of measuring angular velocities and linear accelerations, normally provides needed information such as joint angles. Its measurements are proven clinically accurate, reliable and cost effective; however, clinicians or researchers may be unfamiliar with the IMU device; and it may not be accessible to many households. We propose a Nintendo Wii-based alternative to measure knee joint angles. Four Wii remotes with Wii Motion Plus were wrapped with elastic tubular bandages on a normal subject, two on thighs and two on shanks (left and right). The device reference frame was set with respect to the anatomical reference frame of the subject. When a subject walked on a treadmill, a knee joint angle was calculated from the difference between shank and thigh angles obtained from gyroscope angular velocities. A single static calibration at the beginning of the experiment using and the linear de-drift algorithm were used to eliminate integration errors in every gait cycle. Our method qualitatively exhibits knee angle graphs consistent with other measurements, which it could be an alternative for a knee joint measurement.

Keywords: *inertial measurement unit, knee joint angle, Nintendo Wii*

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Effect of simulated gastrointestinal digestion on the ACE inhibitory activity of peptides derived from Thai Hom-Mali rice bran

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Abstract

Food-derived bioactive peptides with Angiotensin-converting enzyme inhibitory (ACEI) properties are receiving special attention due to their beneficial effects in the treatment of hypertension. In this work we evaluated the impact of a simulated gastrointestinal digestion on the stability and activity of peptides derived from Thai Hom-Mali rice bran. The enzymatic protein hydrolysates were subjected to ultrafiltration using membranes with cutoffs of 10 and 50 kDa in order to obtain products with a narrower range of molecular size. The hydrolysates from rice bran proteins and their fractions (non-ultrafiltration (NU), permeate of 50 kDa (P50), and retentate of 10 kDa (R10)) showed an increase in ACEI activity (50% inhibitory concentration [IC_{50}] = 101.67, 53.82, and 7.62 μg of protein/mL, respectively) when the peptide size was decreased. After *in vitro* gastrointestinal digestion (pepsin and pancreatin), a significant increase in the ACEI potency of the rice bran peptides was observed (IC_{50} = 11.14 and 26.66 μg of protein/mL for NU and P50, respectively) except for the R10 (6.69 μg of protein/mL). The stability of all peptide fractions to the Angiotensin-converting enzyme before and after simulated gastrointestinal (SG) digestion was also revealed. Without SG digestion, the IC_{50} of all peptides considerably increased after incubating with ACE indicating that they are *the substrate type* peptides. Contrarily, the IC_{50} of all SG digested peptides against ACE were not significantly changed demonstrating *the pro-drug type* ACEI peptides. Based on their remarkable antihypertensive activity, peptides from Thai Hom-Mali rice bran may have potential applications as nutraceutical compounds in functional foods.

Keywords: *Thai Hom-Mali rice bran peptides, ACE inhibitory activity, ultrafiltration, simulated gastrointestinal digestion*

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Relationship between von Willebrand factor, ABO blood group and coronary stenosis in coronary artery disease

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Von Willebrand factor (vWF), a multimeric glycoprotein which essential for platelet aggregation under the high fluid shear stress as in arterial stenosis. Its levels in plasma are varying significantly within and between individuals. These variations have been associated with ABO blood group. Moreover, high vWF levels are associated with an increased risk of coronary artery disease (CAD). The purpose of this study was to investigate the association between vWF, ABO blood group and the numbers of coronary artery stenotic lesions in CAD. The concentration of plasma vWF was measured by an in-house enzyme-linked immuoserbent assay (ELISA) in 159 patients who were clinically suspected of having CAD undergoing coronary angiography at Queen Sirikit Heart Center of the Northeast Hospital, Khon Kaen University. Based on angiographic results, 97 patients were classified as CAD and 62 patients were non-CAD. Subjects with non-O blood group had significantly higher plasma vWF levels than in group O individuals (0.90±0.39 vs. 0.70±0.38 IU/mL, p=0.002). Slightly elevated levels of vWF were found in CAD group compared to non-CAD group (0.90±0.42 vs. 0.83±0.44 IU/mL, p=0.290). An increased vWF antigen was also observed in patients with multi-vessel disease compared to individuals with single-vessel disease (0.94±0.41 vs. 0.83±0.43 IU/mL). However, the difference was not significant (p=0.117). In contrast, the significantly increased plasma vWF levels were found in group O patients with multi-vessel disease (0.85±0.41 vs. 0.55±0.27 IU/mL, p=0.002). In non-CAD group, plasma vWF was significantly higher in group non-O than in group O (0.90±0.39 vs. 0.52±0.22 IU/mL, p<0.001). In conclusion, this study confirmed the relationship between ABO blood group and vWF plasma levels and suggested that vWF levels seem to be associated with CAD. However, it was not a major determinant to develop coronary stenosis in CAD.

Keywords: *coronary artery disease, coronary stenosis, von Willebrand Factor, ABO blood group*

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Vasorelaxant effect of *Bacopa monnieri* extract on rat isolated pulmonary artery

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Bacopa monnieri (Brahmi) is a medicinal plant traditionally used for several conditions including respiratory and cardiotonic disorders. Since Brahmi extract has vasorelaxant effects on isolated aorta in rabbit and rat, we argued that respiratory distress through pulmonary hypertension might be alleviated by Brahmi by pulmonary vasodilatation. Thus, the present study aimed to investigate possible mechanism of Brahmi ethanolic extract on isolated segments of rat pulmonary artery *in vitro*. When pre-contracted with 10^{-5}M phenylephrine (PE), Brahmi extract (0.05-4.0 mg/ml) induced concentration-dependent relaxations on both endothelium intact (IC_{50}, 1.426±0.41mg/ml) and denuded ($IC_{50,}$ 1.018±0.41 mg/ml) vessels. Pre-incubation of endothelium-denuded pulmonary artery in Ca-free medium with either 10^{-6}M nifedipine (to block L-type Ca^{2+} channel blocker) or Brahmi extract (0.5, 2.5 and 4.0 mg/ml) for 10 minutes abolished or reduced (25±5.24, 61±5.93 and 83±6.19 %) vasoconstriction induced by $CaCl_2$ (0.01-10mM), respectively. This study showed that Brahmi extract promoted similar vasorelaxant effects of pulmonary artery in endothelium-intact and denuded, suggesting that Brahmi extract produced endothelium-independent vasorelaxation. Brahmi extract probably acted directly on vascular smooth muscle cells. In addition, Brahmi extract showed concentration-dependent rightward shift of $CaCl_2$-induced contraction, whereas nifedipine completely abolished these contractions. These indicated that Brahmi extract inhibited Ca^{2+} influx into vascular smooth muscle cells. Our results provided functional evidence that the vasorelaxant effects of Brahmi extract on pulmonary artery vascular smooth muscle cells.

Keywords: *Bacopa monnieri, vasorelaxation, pulmonary artery, Ca^{2+}*

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Networking improves accessibility of stroke fast track treatment at Srinagarind Hospital, Khon Kaen, Thailand

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Stroke Fast Track was established at Srinagarind Hospital May 1, 2008. The treatment costs are supported by the National Health Security Office (NHSO). We launched a stroke fast track campaign to the community and local hospital around our tertiary hospital in October 2009. The aim of our project was to study the successfulness of stroke networking on stroke treatment and the outcome of the stroke fast track program. Since 2008, there 685 patients were diagnosed with acute stroke; of whom 105 received rt-PA treatment (15.3%). In the pre-networking era, 261 patients had been diagnosed with acute stroke; 79 of whom were eligible for stroke fast track (30.3%) and 26 received rt-PA (9.96%) with the average door-to-needle time being 87 minutes. Two patients had intracerebral hemorrhage (7.7%). After the networking campaign, 424 patients were diagnosed with acute stroke; of whom 202 (47.6%) were eligible for stroke fast track and the rt-PA treatment rate was 18.6% (79 patients). The average door-to-needle time was 54 minutes and two patients had intracerebral hemorrhage (2.5%). The overall outcome of stroke fast track may not be different from other reports, but our eligibility rate was higher. In the second year after implementation of the stroke fast track service outcomes were better and there was a higher rate of participation. In addition to technical experience, the networking campaign may play an important role on the successful outcomes. These results may support the importance of the networking campaign for better outcomes vis-à-vis facility's awareness and patient accessibility.

Keywords: *stroke fast track, rt-PA, stroke networking, stroke*

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