THE 33rd ANNUAL ACADEMIC MEETING OF THE PHYSIOLOGICAL SOCIETY OF THAILAND

Abstracts of CRN communication
**LC 1:** Carnitine Transport in The Male Reproductive Tract: A Target for Male Contraception
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Carnitine, 3-hydroxy-4-trimethylamino butyric acid, is present in all animal tissues and has an essential role in the metabolism of long chain fatty acids. The major source of carnitine is dietary intake although some tissues such as liver can synthesize it. However, the male reproductive tract especially the epididymis contains the highest levels. Its concentration in the cauda epididymal fluid is about 2,000 times that in the blood. Several lines of evidence suggest that carnitine in the epididymis plays an important role in sperm motility and male fertility. In spite of this fact, the mechanism of carnitine transport in the epididymis is still not clearly understood. Studies using luminal microperfusion of the epididymis both in vivo and in vitro showed that transepithelial fluxes exhibit saturable kinetics and specificity for carnitine related compounds. Tissue uptake studies in vitro using oil-filled tubules of the rat distal caput revealed two different pathways for carnitine uptake at the basolateral membrane; 1) a saturable and Na\(^+\)-dependent, 2) a non-saturable and Na\(^+\)-independent. Similarly, the effluxes from the preloaded tubules consisted of two components, both are saturable and interact differently with carnitine analogs.

Recently, several transporters of carnitine have been identified and cloned from rat small intestine (CT1), human kidney (OCTN2), and human testis (CT2). All are Na\(^+\)-dependent with high affinity to carnitine and show no stereospecificity. It appears, however, that CT1 resembles OCTN2, but not CT2. The latter belongs to a new family phylogenetically located between the organic cation transporter (OCT/OCTNs) and anion transporter (OATs) families. Immunohistochemistry revealed the presence of CT1 on the basolateral membrane and CT1 on the luminal border of the epididymis. Both types of transporter play different roles in the transepithelial transport of carnitine in epididymis. The detail mechanisms by which carnitine is accumulated at high concentration in the lumen require further studies. Nevertheless, the current knowledge on carnitine transporters provides a new avenue for male contraception.

**LC 2:** Estrogen Regulation of Chloride Secretion in immortalized Procine Endometrial Epithelial Cells
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The role of estrogen in regulation of Cl\(^-\) secretion was studied in immortalized porcine endometrial epithelial cells (immortalized PEG cells) using Ussing chamber technique and molecular approaches. Several techniques have been employed to 1) identify and characterize estrogen receptor subtypes using Western blot analysis, 2) investigate the influence of estrogen treatment on uridine triphosphate (UTP)-dependent stimulation of apical Cl\(^-\) current and basolateral K\(^+\) current using amphotericin B-permeabilized monolayers, and 3) identify the estrogen regulation of Ca\(^{2+}\)-activated SK3 channel expression using RT-PCR. The preliminary results demonstrated the presence of estrogen receptor alpha and beta subtypes in immortalized PEG cells. Previous studies have shown that UTP stimulate two distinct Cl\(^-\) channels, Ca\(^{2+}\)-activated Cl\(^-\) channels and PKC-activated Cl\(^-\) channels. In the present results, estrogen treatment produced a significant increase in UTP-stimulated Cl\(^-\) current with no significant change in both component of Cl\(^-\) current. Estrogen was also found to stimulate basolateral K\(^+\) current. Finally, estrogen was shown to upregulate the expression of SK3 channels. Taken together, the preliminary results suggest that, in immortalized PEG cells, estrogen may likely stimulate the basolateral K\(^+\) channel, possibly via SK3 channel,
which provides a driving force for Cl− secretion. However, many studies are required for clarification of the transport-related regulatory mechanisms of estrogen on endometrium epithelium.

**LC 3:** Detection of Cytochrome P450s Genetic Polymorphisms
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Professor Tetsuya Kamataki, The director of The Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Science, Hokkaido University, kindly arranged for my visiting and training in the field of molecular genetics especially the techniques for detection of cytochrome P4502A6 (CYP 2A6) genetic polymorphisms. Under the guidance of Assist. Prof. Dr. Masaki Fujieda and Dr. Masaharu Sakurai, total of 25 DNA samples were extracted from peripheral lymphocytes of Japanese lung cancer patients and subjected for detection of CYP2A6 allelic variants. By using PCR-based approach coupled with the restriction fragment length polymorphism (RFLP), the CYP2A6 allelic variants can be characterized as CYP2A6*1A (wildtype allele; 42%), CYP2A6*1B (18%) and CYP2A6*4C (whole deletion type; 8%). Allele-specific PCR assays were applied for identification of single nucleotide polymorphism (SNPs) at T1412C (CYP2A6*7) in exon 9 (14%) of the CYP2A6 gene and this variant detection was confirmed by DNA sequencing. Melting point analysis by Real-time PCR was performed in DNA samples which have one or two CYP2A6*1A alleles for characterization of CYP2A6*9 (SNP at T-48G in TATA box; 18%).

These molecular techniques were introduced into the 21st International Pathophysiology Symposium and Workshop on Health Effects of Heavy Metals: Role of Cytochrome P450s Genetic Polymorphism on Metal Toxicity and Hypertension during April 2-5, 2003 in Chiang Mai Province which an overall 18 scientists had participated in the workshop. Until now these techniques have also been extended for training our graduate students.

The other optional techniques I had been trained from Professor Kamataki’s lab were the isolation of mRNA and RT-PCR for gene expression study.

**LC 4:** Electrophysiological recording of Hippocampal Synaptic Transmission In Vivo
Roysommuti S
Department of Physiology, Faculty of medicine, Khon Kaen University, Khon Kaen 40002, Thailand
Abstract is not available.

**LC 5:** Brain and Neurological development
Muchimapura S
Abstract is not available.

**LC 6:** Visit at Toyama Medical and Phamaceutical University and Okazaki National Research Institutes, Japan
Tilokskulchai K and Taphem S
Department of Physiology, Faculty of medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
Abstract is not available.
LC 7: Protection of Centrilobular Necrosis by Curcuma comosa: A Study of CCl₄-Induced Hepatotoxicity in Mice
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Background: Hepatitis is one of the most common liver diseases in which chronic inflammation would result in liver injury, structural damage, fibrosis and cirrhosis. Treatment of these diseases is difficult and less effective. Therefore, patients are often interested in using herbal products as an alternative medicine. Currently, a number of plants have been used and believed to have therapeutic benefit. In Thailand, Curcuma comosa Roxb, one of the local plants, is widely used in indigenous medicines for treatment of inflammation in many tissues as well as bile-expelling action. However its anti-inflammatory action at the liver has not yet been evaluated. The present study aims to determine whether the extract of C. comosa has any protective activity against CCl₄-induced hepatotoxicity.

Methods: CCl₄ at various doses were intraperitoneally injected into adult Swiss albino male mice and the hepatotoxicities were evaluated after CCl₄ administration. An optimum dose of CCl₄ was chosen for further studies. The protective effect of the hexane extract of C. comosa was evaluated by an intraperitoneal injection of the extract at various periods prior to CCl₄ treatment.

Results and conclusion: Administration of CCl₄ induced dose-dependent changes the morphological and histological features of liver. The degenerative changes of hepatic parenchyma occurred at the area surrounding the central vein and some were extended into the midzonal regions. A single administration of the hexane extract of C. comosa for 12 to 24 hours prior to CCl₄ effectively prevented the CCl₄-induced elevations of several hepatotoxic markers in plasma including plasma glutamic pyruvic transaminase and plasma glutamic oxaloacetic transaminase activities. Examination of liver histology revealed the centrilobular necrosis, which is a typical characteristic of CCl₄-induced hepatic injuries, was not detected in the extract-pretreated group at 24 h. The possible mechanisms by which C. comosa protected hepatic injuries from CCl₄ were then investigated. It may relate to a decrease in CCl₄ bio-activation and also an acceleration of the detoxifying mechanisms to scavenging of the CCl₄ metabolite, trichloromethyl free radical.

LC 8: Methods of studying the regulation of gastric secretion
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Training in the topic of “the regulation of gastric secretion” was held in the laboratory of Professor Catherine Chew at Medical College of Georgia, Augusta, Georgia, USA between May 30, 2002 and November 27, 2002. The training program composed of the following topic:
a) Radiation safety training program, which is required for everyone who wants to work with radioactive substances in research.
b) Study the occupational health considerations for animal users by online training, which is requirement for all animal users.
c) Isolation of the gastric gland and parietal cells from rabbit gastric mucosa using pronase and collagenase digestion.
d) Determination of gastric acid secretion using ¹⁴C aminopyrine uptake measurement.
e) Determine the effect of Ya-hom on the ¹⁴C aminopyrine uptake of the gastric gland stimulated by histamine and carbachol.

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f) Using immunofluorescence technique and confocal microscope to study cytoskeletal proteins.
g) Study the intracellular calcium level in parietal cell using Fura-2 as a fluorescence probe.
h) Construction the plasmid LIM-HA DNA with the vector PCDNA3 and transfekt in parietal cells.

This training program provides the experience in the separation technique and the methodology for studying the functions of gastric glands and parietal cells.

**LC 9:** The Prokinetic effects of Barakol in Rat Intestine
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Barakol, an active constituent extracted from the plant Cassia siamea, has been shown to inhibit dopamine release from brain striatum. In enteric nervous system, an increase in intestinal motility by prokinetic drugs is mediated by increasing cholinergic of decreasing dopaminergic activities. This study was to investigate the possible effect of barakol on spontaneous and neurotransmitter-mediated contraction of longitudinal smooth muscle in the isolated rat ileum. Barakol (10 µM – 1 mM) increased atropine-sensitive isometric contractions in dose dependent manner. Barakol (10 µM – 100 µM) stimulated whereas barakol (>100 µM) inhibited the saxitoxin- and aropine-sensitive contraction elicited by electrical field stimulation (EFS; 20 Hz, 50 V, 100 sec duration). The inhibitory effects of norepinephrine (NE, 10 µM) on the EFS-elicited contraction but not on the spontaneous contraction were abolished in the tissues pretreated with barakol. Dopamine-inhibited muscle contraction was not affected by barakol pretreatment. These results demonstrate the direct stimulatory effect of barakol on spontaneous and neuronally mediated smooth muscle contraction in isolated rat ileum and its antagonistic effect on the activity of adrenergic myenteric neurons. These findings may provide data for clinical application of barakol as a potential prokinetic drug.

**LC 10:** Effect of royal jelly to lipid peroxidation in diabetic rats
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Royal jelly is a thick milky product secreted from hypopharyngeal (cephalic) and mandibular glands of young worker bees (Apis mellifera Linne.). Little has been know about the antioxidant activity (1, 2). In order to investigate the antioxidant effect of royal jelly, diabetic Wistar rats were induced by intravenous administration of streptozotocin, 50 mg/kg. Serum glucose, malondialdehyde (MDA), glutathione, superoxide dismutase (SOD), catalase and glutathione peroxidase activities were monitored throughout the experiments. The very high level of serum glucose (499.33 ± 20.56 mg%) compared to the control group (166.00 ± 19.95 mg%) was found to confirm hyperglycemic condition. Serum MDA level was also high in diabetic rat and significantly different (p < 0.001) when compared to the control group.

In the post-treatment groups, lyophilized royal jelly at 2.0 g/kg body weight/day was administered orally for 8 weeks in the following day after streptozotocin injection. It was found that royal jelly significantly decreased (p < 0.001) serum MDA in diabetic rat after 4 week of diabetic induction. For the pre-treatment groups, the same dose of lyophilized royal jelly was administered.
orally 2 weeks before streptozotocin injection. Royal jelly also significantly decreased (p < 0.001) serum MDA in diabetic rat after 4 week of diabetic induction. In both pre- and post-treatment groups, the glutathione, SOD, catalase and glutathione peroxidase activities were not significantly changed from the control groups. The indirect measurements of free radicals (MDA) indicated the action of royal jelly to inhibit lipid peroxide formation in diabetic rats.

LC 11: Increased glycation is the mechanism of impaired glucose-induced insulin secretion from DBA/2 islets in prolonged high glucose culture.

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Background: Type 2 diabetes is characterised by basal hyperinsulinaemia and impaired glucose-mediated insulin secretion. This state of increased basal insulin secretion and decreased glucose-induced insulin secretion can be reproduced in vitro by prolonged exposure of islets to a high glucose concentration. It has been hypothesized that this defect is caused by glucose toxicity and/or β-cell exhaustion. However, the exact mechanism of this defect is not well understood. Genetic predisposition might play an important role in the deterioration of islet function after exposure to a sustained excessive glucose milieu. The DBA/2 (D2) mouse is a model of pancreatic islet failure and becomes diabetic when chronically challenged with a high glucose environment. The mechanism for diminished insulin secretion in the D2 mouse is not known.

Aims: To determine what is/are the biochemical step(s) that cause(s) impaired insulin secretion after prolonged culture of DBA/2 islets in high glucose.

Methods: Pancreatic islets were isolated from D2 and C57BL/6 (B6) control mice by collagenase digestion and cultured in 11.1 mM for 10 days or 40 mM glucose for 10 days with or without 1 mM aminoguanidine. Insulin release (mU.L⁻¹/5 islets. 60 min) was assessed after 60 min incubation with glucose at 2.8 and 20 mM. Levels of glucokinase (Gk), hexokinase (Hk) and Glut2 were measured using standard biochemical techniques.

Results: While islet insulin content was decreased following 40 mM glucose culture in both strains, there was no difference between D2 and B6 islets at either 11.1 mM (3403 ± 210 vs 3770 ± 497 mU/5 islets) or 40 mM glucose culture (967± 116 vs 815 ± 106 mU/5 islets). When insulin secretory function was expressed as percent increase of 20 mM vs 2.8 mM glucose, there was no difference between D2 and B6 islets (470 ± 90 vs 440 ± 150) following culture at 11.1 mM glucose for 10 days. In contrast, the increase in insulin release was significantly lower in D2 compared to B6 islets (216 ± 33 vs 360 ± 60, P<0.05) following culture at 40 mM for 10 days. This profound impairment in glucose-mediated insulin secretion in D2 islets was associated with a significant decrease in the activity of the rate-determining enzyme, Gk (3.2 ± 0.7 vs.5.7 ± 2.0 nmol/min/mg protein, P<0.05). Furthermore, Western blot analysis showed a significantly lower level of Gk protein in D2 compared to B6 islets (24 ± 6 vs. 36 ± 5 arbitrary densitometric units, P<0.05), while there was no difference in the levels of Hk (61 ± 20 vs. 80 ± 27 arbitrary units) or Glut2 protein (95± 35 vs. 88 ± 45 arbitrary units). It has been suggested that chronic hyperglycemia might change glucokinase activity through a process of non-enzymatic glycation. After co-culture islets at 40 mM glucose for 10 days with 1 mM aminoguanidine (AG), a well know glycation inhibitor drug, the fold increase (of 2.8 mM) of glucose-induced insulin secretion were restored in D2 islets (4.0 ± 0.5 vs 2.2 ± 0.3). Also, Western blot analysis demonstrated that there was a significant increase in the amount of glucokinase protein in D2 islet co-cultured with AG compared to D2 islet cultured
with 40 mM glucose alone (57 ± 3 vs. 24 ± 6 arbitrary densitometric units). AG had no effect on glucokinase protein levels in B6 islets.

Conclusions: In summary, this study showed that chronic exposure of D2 islets to 40 mM glucose produced a defect in insulin secretion compared to B6 islets, and that this was due to decreased islet glucokinase activity and protein levels. The cause of decreased glucokinase activity in D2 islets in response to high glucose environment was associated with increased glycation. Our study suggested that advanced glycation end products might play a role in inhibiting glucokinase activity, which leads to reduced

**LC 12:** Antioxidant activities of the extracts from the leaves of Ocimum sanctum Linn.
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The powdered leaves of Ocimum sanctum Linn. were extracted by hexane, chloroform, ethanol and water. All of the extracts were tested for antioxidant potency using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The 50% inhibition of DPPH was used to interpret the antioxidant effect (1). Hexane and chloroform extracts did not show 50% inhibition. Ethanol extract showed 50% inhibition at high concentration, more than 1,400 µg/ml. The best fraction was aqueous extract expressed 50% inhibition at 324.21 ± 29.13 µg/ml. Total antioxidant status was also investigated by the suppression of radical cation \( \text{ABTS}^{••} \) produced from the reaction of \( \text{ABTS}^{•} \), peroxidase and hydrogen peroxide (2). Hexane extract had no antioxidant activity. The total antioxidant activities of chloroform, ethanol and aqueous extracts at 150 µg/ml were 0.47, 0.83 and 1.05 mmol/L respectively. In the comparison to standard antioxidant, BHT showed 16 times higher antioxidant activity than aqueous extract in both assays. Base on these findings, the leaves of Ocimum sanctum should contain the antioxidant constituents with high polarity. The aqueous extract will be useful for further investigation.

**LC 13:** Antidiabetic effect of Abutilon indicum
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Objective: To train some techniques for research about Thai plants that are confirmed to have anti-diabetic or hypoglycemic effects in animal model.


Background: Thai medicinal plants; 'Abutilon indicum leaf, 'Abutilon indicum seed, and Pandanus odorus rhizome, which were tested the anti-diabetic or hypoglycemic effects in rats by glucose tolerance test. They were further studied to find fatty acid composition which are the data for basic lipid knowledge of plant. The lipid transfer proteins (LTPs), which are the plant specific protein defined by their ability to facilitate transfer of phospholipids between membrane in vitro, were also studied. Some proposed biological roles of LTPs are anti-fungals, anti-diabetics and etc.

Material and Methods: Fresh plants were collected in Thailand. They were boiled for 5 min and carried to study lipid composition and LTP activity in France. For lipid composition, plant lipid were extracted and separated by TLC with Lapage solution and injected in Gas chromatography.
For LTP purification, plants were homogenized with 400 mM sucrose, 1 mM Na₄EDTA, 1 mM cysteine chloride, 10 mM beta-mercaptoethanol, 100 mM tris-HCl (pH 7.2). The homogenate was filtered and centrifuged. The mixture was stirred overnight and centrifuged. The protein pellet was suspended in 10 mM potassium phosphate (pH 7.2), 30 mM KCl, 8 mM beta-mercaptoethanol. After dialysis, denatured proteins were removed. The supernatant was designated as the protein extract. The proteins were blotted in non dissociated polyacrylamide gel electrophoresis and transferred to cellulose plate. The cellulose plate was bathed in 2 kinds of LTP antibody from Maiz and Rape seed to find whether there is LTP in plant extract or not.

Results and conclusion: Fatty acid composition (%w/w) in Thai plants: Abutilon indicum leaf (C16:0=20.8, C16:1=5.7,C16:2=2.6,C16:3=1.3,C18:0=2.3, C18:1=1.6, C18:2=11.4,C18:3=54.2), Abutilon indicum seed (C16:0=27.7, C16:3=3.6,C18:0=12.3, C18:1=2.7, C18:2=33.0,C18:3=11.2, C20:2=2.1, C22:2=7.3), Pandanus odorus rhizome (C16:0=25.1, C16:1=0.6,C16:2=1.4, C16:3=0.6,C18:0=7.0, C18:1=7.5, C18:2=44.9,C18:3=10.3, C20:2=0.9, C20:1=0.7, C20:3=1.1). For LTP study, it is found that LTP Ab from Maiz could bind with some LTP proteins in Abutilon indicum leaf (+), Abutilon indicum seed (+++) and Pandanus odorus rhizome (+) but not in Pandanus odorus leaf (control, without hypoglycemic activity) and LTP Ab from Raphe seed could not attach any protein. It showed the presence of LTP in Abutilon indicum leaf, Abutilon indicum seed and Pandanus odorus rhizome which should be further worked on to find LTP activity in relation with the anti-diabetic activity.

LC 14: The effect of a Thai herbal medicine on cerebral activity detected by functional magnetic resonance imaging in the rat
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Background: Thunbergia laurifolia Linn (TH) is a herbal medicines used as an antidote for several poisonous agents in Thai traditional medicine. This plant has also been used extensively as component of mixture of crude extracts to treat patients with drug addiction (1) but there are no clinical trials on the use of TH in the treatment of drug addiction. Recently, we demonstrated that amphetamine and TH significantly increased potassium-stimulated dopamine release from rat striatal slices when compared to potassium-stimulated alone. TH potentiated the effect of amphetamine on potassium-stimulated dopamine release when compared to amphetamine alone. The results indicate that TH may stimulate dopamine release in the same manner as amphetamine (2).

Objective: This study aims to test the effect TH on cerebral activity detected by functional magnetic resonance imaging (fMRI) in the rat

Material and method: fMRI was performed on a 2.35T Bruker Biospec Advance MR System. Consecutive single slice functional imaging over the whole of the rat brain was performed to investigate the changes in signal intensity at various parts of the brains induced by TH (200 mg/kg) or vehicle action.

Results: The fMRI study demonstrated that TH increases % signal intensity at various areas in the brains such as amygdaloid nuclei, parietal cortex, frontal cortex, nucleus accumbens, caudate putamen and hippocampus.
Conclusion: The result was the first to indicate that TH activates various parts of brains responsible for reward and locomotor similar to the effect of amphetamine and cocaine previously reported (3). Further studies are required to investigate the value of the plant for the treatment of drug addiction.

LC 15: Effects of Ischemia on Rat Brain as a Model for Aged Brain
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Ischemia in certain brain regions causes clinical symptoms which are similar to symptoms of dementia due to old age. In the present study, to explore these similarities, we examined changes induced by ischemia in the hippocampus and neocortex in rats. Three week old rats were divided into five groups: both common carotid ligatures, unilateral ligature, (left or right), 10 and 20 minute bilateral occlusions, sham operation, and positive control (liver or brain injury). The rats were sacrificed on day 7 after the operations. Tissue samples were taken, fixed, embedded in paraffin. Histochemical technique was applied with diluted lectins. We investigated whether the 11 selected types of lectins would give a different staining on the hippocampus and neocortex slices obtained from the hypoxic rat brains. The apoptotic lesions of the brains were also detected by comparing them with those of the positive control. We found that some lectins used gave some different patterns of staining in hypoxic rat brain slices when compared with those from the sham group. Further investigation must be done using selected lectins to confirm if there are some specific patterns of staining in the specific regions of the brain lesions.

LC 16: Study on methods & techniques to investigate the anti-cancer activity of medicinal plant
Thongpraditchote S
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I was accepted as a scientific visitor in Professor Ikuo Saiki’s laboratory, Department of Pathogenic Biochemistry, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, for a period of 7 days (during August, 24th - August, 30th, 2003) under the fund from CRN in Physiology.

In this laboratory, they are investigating the control of cancer invasion and metastasis, tumor-induced angiogenesis, and immunological diseases by various treatment modalities including natural products, medicinal plants and traditional medicines in animal models. They are also studying the regulatory mechanism of cell functions and signal transduction.

LC 17: Kinetic Analysis of Rhodamines Efflux Mediated by the Multidrug Resistance Proteine (MRP1)
Saengkhae C, Loetchutinat C and Arlette-Suillerot
Laboratoire de Physicochimie et Cellulaire, Univresité Paris Nord, Bobigny, France

Characterization of rhodamine 123 as functional assay for MDR has been primarily focused on P-glycoprotein-mediated MDR. Several studies have suggested that Rh123 is also a substrate for MRP1. However, no quantitative studies of the MRP1-mediated efflux of rhodamines have, up to now, been performed. Measurement of the kinetic characteristics of substrate transport is a powerful approach to enhancing our understanding of their function and mechanism. In the present study, we
have used a continuous fluorescence assay with four rhodamines dyes (rhodamines 6G, tetramethylrosamine, tetramethylrhodamine ethyl ester and tetramethylrhodamine methyl ester) to quantify drug transport by MRP1 in living GLC4/ADR cells. The formation of a substrate concentration gradient was observed. MRP1-mediated transport of rhodamine was glutathione-dependent. The kinetic parameter, $k_a = V_m/k_m$, was very similar for the four rhodamines analogs but 10-fold less than the values of the same parameter determined previously for the MRP1-mediated efflux of anthracycline. The findings presented here are the first to show quantitative information about the kinetics parameters for MRP1-mediated efflux of rhodamine dyes.

**LC 18**: Techniques in Direct and Indirect Tests of Diaphragm Strength
Khrisanapant W
Abstract is not available.

**LC 19**: A Report of the Research Training and Teaching Observation in Exercise Physiology at the University of Melbourne, Australia
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This report was mentioned on research training and teaching observation in exercise physiology as well as the other experiences during the training period. The kind host of this training was Dr. G McConell and his group, The Human Exercise Physiology, Department of Physiology, University of Melbourne. The reporter most involved with a study on the effect of hypoxia on skeletal muscle AMPK activity during exercise. The reporter participated in two other studies: Dr. G. Wadley, a postdoctoral fellow’s study on the effect of L-Arginine infusion on glucose kinetics during exercise in humans and that of Mr. R. Berg, a PhD student on the effect of exercise training on skeletal muscle AMPK activity during exercise. The group specialized in exercise metabolism. The reporter saw and was involved only in getting samples (intravenous cannulation and muscle biopsy) and recording the exercise performance values from subjects. Rarely on the biochemical assays due to the time limitations. The reporter attended various seminars arranged by the Group, Department, Faculty and the Centre. The observations of teaching exercise physiology were mainly at the School of Health Sciences, Deakin University and some exercise related courses at the Department of Physiology, University of Melbourne. PBL classes for second year medical students were also attended. The problems, suggestions and the perspectives from this training were included in this report. This training was financially supported by the CRN in Physiology, The Ministry of University Affairs, Thailand.

**LC 20**: Visit at Department of Pharmacology, Faculty of Medicine, Dentistry, and Health Sciences, The University of Melbourne, Melbourne, Australia 16-30 August 2003
Thirawarapan S and Suvithayawut W
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The purpose of the visit were to observe and discuss the research work involved the animal models and cell culture techniques in studying atherosclerosis, and to make a research collaboration for the Ph.D. student who received the study grant under CRN (Physiology), year 2002.

The animal models, induced to develop thrombosis, atherosclerosis and hypertension, are used in studying the abnormal cardiovascular function and the effect of drugs as well as substances
from plant extract. They have a collaborative research in Howard Florey Institute studying atherosclerosis using Apo-E def mice, both in vivo and isolated cells. The vascular functions also study in isolated arterial preparations (aortic, carotid and mesenteric arteries).

The obesity and hyperglycemic rats are used to study the secretion of neurotransmitters/neuropeptides, such as neuropeptide Y and leptin, in the brain. The eating behavior effect of MSH and galanin by intracranial injection also study in these metabolic syndrome animals.
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Abstracts of free communication
L 1: State-Dependent Release of Glycine and GABA in Clarke's Column of the Upper Lumbar Spinal Cord
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Evidence now exists that proprioceptive sensory information conveyed via the dorsal spinocerebellar tract (DSCT) is subjected to dynamic suppressor influences during REM (desynchronized or active) sleep. We recently reported that microiontophoretically administered strychnine or bicuculline markedly reduced the REM sleep-related suppression of spontaneous spike activity of DSCT neurons suggesting that glycine and GABA inhibits these neurons during REM sleep. Accordingly, the present study employed microdialysis techniques to measure state-dependent release of glycine and GABA in Clarke’s column in 2 cats. The location of Clarke’s column at L₃ was confirmed by recording cerebellar evoked field potentials. The recording electrode was carefully replaced with a dialysis probe at the same depths where maximal amplitude field potentials were recorded. The animal’s behavioral state was continuously monitored and dialysis samples (10 microliters) collected during different behavioral states. Glycine and GABA levels in the perfusate were determined by HPLC with fluorescent detection. Data obtained across 19 sleep wake cycles indicate glycine levels were increased in Clarke’s column by ~40% during REM sleep when compared to preceding episodes of wakefulness. There was no significant difference in the levels of glycine between the states of wakefulness, quiet sleep or awakening from REM sleep. GABA levels measured from 12 sleep cycles also demonstrated a similar profile and increased by ~34% during REM sleep when compared to preceding episodes of wakefulness. These data provide additional support for the idea that dynamic inhibition during REM sleep contributes to the reduction of sensory throughput to higher brain centers conveyed via the DSCT.

L 2: Detectable Cardiac Troponin T Plasma Concentration in Siamese Fighting Cocks: A Possible Model of Stress – Induced Myocardial Injury
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¹Department of Physiology, ²Department of Pathology, Veterinary Medicine, ³Department of Animal Science, Faculty Agriculture, Kasetsart University, Bangkok 10900, Thailand
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Objectives: To test the hypothesis of stress-induced cardiac injury in the fighting cocks.
Background: Several physical and emotional factors have been reported to be associated with variations in levels of blood biochemical parameters in avian species. The fighting cocks are presumed to be naturally under acute and chronic “fight and flight” stress situation.
Material and Methods: Measurements of the conventional cardiac enzymes aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and its isoenzyme CK-MB as well as the novel cardiac-specific marker troponin T (cTnT) were performed on 35 Siamese fighting cocks.
Results: The results revealed that compared to female fowls (n=35), the fighting cocks exhibited significantly higher activities of all cardiac enzymes measured. The male fowls also showed a significantly higher cTnT plasma level than female fowls. Detectable or increased cTnT concentrations were seen in nearly half of the male but only in one out of 35 female animals.
Significant correlations between cTnT and CK, AST and LDH, but not CK-MB, were observed in the male fowls. Conclusion: These results indicate that stress-induced cardiac damage as evidenced by detectable cTnT concentrations exist in the fighting cocks. This avian species may thus provide a useful model for studying myocardial injury caused by stress situation.

L3: The Involvement of K\(^+\) Channels in The Vasorelaxant Responses of The Rat Arteries to Testosterone
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Previous studies have demonstrated that testosterone induces vasorelaxation via activation of K\(^+\) channels [1,2]. However, the exact types of K\(^+\) channels, which mediate the testosterone-induced vasorelaxation are still unclear. The aim of this study was to investigate the role of K\(^+\) channels in testosterone-induced vasorelaxation in the rat arteries.

The thoracic aortae and mesenteric arterial beds were dissected from the male Wistar rats. The vessels were bathed or perfused with oxygenated Krebs-Henseleit solution. Following a 1-hour equilibration period, methoxamine was added to induce contraction. Testosterone was added cumulatively to the perfusion fluid. The vasorelaxant effects of testosterone were assessed in the presence of 10\(\mu\)M indomethacin, 100nM charybdotoxin (ChTx), 1mM 4-aminopyridine (4-AP), 10\(\mu\)M glibenclamide, or 30\(\mu\)M barium chloride (BaCl\(_2\)). To examine the effects of high extracellular K\(^+\) on responses to testosterone, 30 and 60mM KCl were added to induce tone. Data were compared by the Student’s unpaired t-test or ANOVA.

In the presence of indomethacin, testosterone induced acute concentration-related vasorelaxation in both aortic rings and mesenteric arterial beds. In the aortae, high KCl or ChTx significantly (p<0.001) reduced the potency of testosterone-induced vasorelaxation at its high potency site. However, testosterone-induced vasorelaxation was not affected by 4-AP, glibenclamide, or BaCl\(_2\). In mesenteric arterial beds, the vasorelaxant responses to testosterone were inhibited by high KCl, TBA and ChTx, but not by 4-AP and glibenclamide.

The present study has shown in rat aortic rings and mesenteric arterial beds that, following blockade of the cyclooxygenase pathway, testosterone-induced vasorelaxation is mediated via activation of K\(_{Ca}\) channels.

L4: The Estrogenic Effects of White Kwao Krue (Pueraria mirifica) on Menstrual Cycle and Hormone-Related Ovarian Function in Premenopausal Monkeys
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White Kwao Krue (WK) is a leguminous plant containing phytoestrogens. We investigated the effects of WK on changes of menstrual cycle and hormone related-ovarian function in premenopausal cynomolgus monkeys (Macaca fascicularis). The monkeys were divided into 2 groups for acute and long-term treatment. In each treatment, they were sub-divided into 3 groups and fed with 10, 100 and 1,000 mg of WK, respectively. The acute treatment, monkeys were fed
with WK for 1 time. The long-term treatment, monkeys were fed daily with WK for 3 menstrual cycles. They were followed up changes of menstrual cycle and hormone levels for 1 cycle before and 2 cycles after the feeding. The blood samples were collected on days 3, 9-14, 19, 24 and 29 of menstrual cycle and every 10 days until the next menstruation was found. Blood sera were assayed for levels of FSH, LH, inhibin, estradiol, and progesterone. The result showed that a single feeding of 1,000 mg of WK prolonged the menstrual cycles, but no changes in hormonal profiles were found. Long-term feeding of WK prolonged the menstrual cycle in the dosage of 10 and 100 mg/day or completely stop the menstruation in the dosage of 1,000 mg/day. Levels of those 5 hormones were decreased in a dose-dependent manner. Our finding indicated that WK can disturb the menstrual cycle in premenopausal cynomolgus monkeys through the suppression of the folliculogenesis and ovulation mechanism, especially for the long-term treatment, it therefore may be applicable for premenopausal women as an alternative contraceptive drug.

L5: Passive Calcium Absorption and Its Response to Prolactin in Suckling Rats
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We have previously observed the enhancing effect of prolactin (PRL) on the intestinal absorption of calcium (Ca) in adolescent and mature rats but not in aged rats. Thus, it was interesting to study the absorption of Ca and its response to prolactin in the suckling rats.

The absorption of 20 mM Ca in the 5 cm intestinal segments was evaluated in 20 day old suckling rats after an acute intraperitoneal dose of PRL or 7 day subcutaneous administration of 2.5 mg PRL/kg body weight. The physiological significance of endogenous PRL was evaluated by inhibiting its secretion by daily administration of 6 mg bromocriptine/kg body weight.

In contrast to the adult rats, PRL had no acute effect on the passive Ca absorption in the suckling rats. However, similar to the adult rats, endogenous PRL increased the lumen to plasma (Ca₅₋₆) flux of Ca in the duodenum and jejunum, but not in the ileum or colon. On the other hand, a high dose of 2.5 mg PRL/kg body weight decreased the duodenal Ca₅₋₆ flux, thus demonstrating the biphasic action of PRL as seen in adult rats. Interestingly, unlike in adult rats, low concentration (40 mM) or an absence of luminal sodium increased the jejunal Ca absorption. In conclusion, our studies demonstrated certain characteristics of calcium absorption in the suckling rats and its response to prolactin which was not exactly the same as in adult rats.

L6: Developmental Potential of Rabbit Nuclear Transplant Embryos from Preimplantation Embryonic and Adult Somatic Cells: Implication for Medical Research Applications (phase 1).
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In animal cloning, metaphase II (M II) oocytes are commonly enucleated blindly by aspirating the first polar body and the adjacent cytoplasm presumably containing the nuclear material. However, the first polar body may sometimes migrated away from the original place of extrusion, so the chromosomes of the oocyte will displace from first polar body. This study was, therefore, carried out to (1) investigate the efficiency of oocyte enucleation and (2) determined the
effect of all kinds of enucleated oocyte treatments on the in vitro development of nuclear transfer embryos.

Matured oocytes were flushed from the oviducts of superovulated matured female rabbits approximately 14-16 hours after hCG administration. The oocytes were randomly divided into 3 groups; In group 1, oocyte enucleation was performed in M II oocytes immediately after collection, group 2, oocytes were preactivated with 5 µg/ml cycloheximide (CHX) + cytochalasin B (CB) for 5 hours before enucleation and group 3, enucleation was performed after aging in vitro for 24 hours. After enucleation all oocytes were incubated in PBS containing 5 µg/ml specific DNA dye for 15 min. at 37 °C to detect the presence of residual chromatin. The oocytes were classified microscopically as enucleated (no chromosomes), partially enucleated (one to several chromosomes remaining), and non-enucleated (with a complete set of chromosomes). In the following experiment, an isolated blastomere of 16-cell stage embryos was transferred into the enucleated oocyte and electrically activated. Development of reconstructed embryos was monitored in vitro.

In the first experiment, staining of DNA with Hoechst 33342 revealed that the oocytes enucleated after chemical preactivation showed a significantly (p<0.05) higher rate of enucleation (90.5%) compared with the non-activated (72.3%) and aged oocytes (61.5%). No significant difference in enucleation rates was observed between the non-activated and aged oocytes (p>0.05), In the second experiment, although the potential of nuclear transferred oocytes develop to blastocyst stage did not differ among the groups, enucleation using in vitro aged oocyte trends to have better supporting than the use of preactivated oocytes.

In conclusion, the result of the present study demonstrated that chemical activation before oocyte enucleation is effective for nuclear transfer in rabbit. But it might have residual effects that may persist during subsequent embryonic development.
THE 33\textsuperscript{rd} ANNUAL ACADEMIC MEETING OF THE PHYSIOLOGICAL SOCIETY OF THAILAND

Abstract: oral session
SC1: Impact of Dietary Fat Proportion on The Endurance Capacity in Trained Rats
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A well-accepted adaptation to consumption of a fat-rich diet is an increased contribution of fat for energy provision. However, whether the different proportion of dietary fat gives rise the similar effects is less well characterized. This experiment was aimed to study endurance capacity, substrate storage and utilization in muscle and liver in rats fed with different percentage of dietary fat. Male Wistar rats were treadmill trained for 10 weeks while consuming normal diet (ND: 11% total energy content (E%) fat) or moderate fat diet (MF: 20E%) or high fat diet (HF: 75E%). At the end of training period rats were killed either before and after endurance run to exhaustion. Increasing percentage of fat in diet led to an increased endurance time compared to normal diet, by 59.33% in MF and by 96.25% in HF (both p<0.05). An analysis of variance revealed that resting TG in the MF and HF was enhanced in muscle (4.1±0.1 and 10.3±0.2 mg/gm tissue, respectively) and liver (12.2±0.5 and 23.9±0.5 mg/gm tissue, respectively) compared with ND (2.1±0.1 and 10.7±0.3 mg/gm tissue in muscle and liver, respectively). Utilization rates of muscle and liver glycogen significantly (p<0.05) decreased while the utilization rates of muscle and liver TG gradually raised as the dietary fat content was increased. These results demonstrated a proportionate increase in endurance capacity with increasing dietary fat which is likely due to an increased ability to oxidize fat and spare muscle glycogen.

SC 2: Role of Respiratory Muscle Strength on Weightlifting Performance in Weight Lifters
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The main purpose of this study was to determine the role of inspiratory and expiratory muscle strength on weightlifting performance in weightlifters. The effect of weightlifting belt on the maximum weight lifted was also investigated.

Young male weightlifters (n=9) of different weightlifting performance and a group of age- and body weight-matched sedentary control (n=9) volunteered as subjects. Inspiratory and expiratory muscle strength were indicated by maximal static inspiratory (P_{Imax}) and expiratory pressure (P_{Emax}), respectively, measured by using a respiratory pressure meter. Spirometric lung function test of lung vital capacity (VC) and forced expiratory volume in 1 sec (FEV₁) was performed in each subject. Comparison of the respiratory muscle strength and lung function between the weightlifters and the control subjects were performed. Weightlifting performance was assessed for three weightlifting types (i.e. snatch, clean and jerk, and isometric) the maximum weight the subject could lift. The results showed that weightlifters had greater P_{Imax} at residual volume (RV) and at functional residual capacity (FRC), and P_{Emax} at total lung capacity (TLC) and at FRC than the control (p<0.05). In the weightlifters, isometric lifting was significantly increased with increased P_{Imax} at FRC (p<0.05) and significantly decreased with increased VC (p<0.05). No significant correlations between P_{Imax} and P_{Emax} and the snatch as well as the clean and jerk weightlifting performance could be found. The VC was significantly decreased by the weightlifting belt (p<0.01). Wearing weightlifting belt could significantly enhance the performance in both the clean and jerk, and the isometric lifting (p<0.01). These results indicate that weightlifters had greater respiratory muscle strength than control. The weightlifters with greater inspiratory muscle strength exhibited higher performance in isometric but not snatch or clean and jerk weightlifting. A decrease in lung volume brought about by wearing weightlifting belt could enhance the weightlifting performance in any
tested subjects. Our findings suggest that inspiratory muscle strength might play an important role on weightlifting performance in low biomechanically dependent weightlifting type. Weightlifting belt could improve the performance in any investigated types of weightlifting.

**SC 3:** Effect of Supplementation on Performance of Thai Collegiate Swimmers

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Creatine has been used to enhance explosive performances in many athletes. However, studies in swimmers showed conflicting results. In addition, there has been no report in Thai swimmers. The present study intends to investigate the effect of creatine supplementation at two differences on body composition and physical fitness of Thai collegiate swimmers and to evaluate the effect of creatine supplementation on swimming performance.

A randomized, double-blind crossover design was employed. Fourteen highly trained swimmers (7 males, 7 females) recruited from top swimming teams of Thammasat and Mahidol university were divided into two groups. Group 1 (n=7) received placebo as the first treatment followed by creatine at low dose (5 g/d), placebo, and creatine at high dose (20 g/d). Group 2 (n=7) received low dose (5 g/d) as the first treatment followed by placebo, creatine at high dose and placebo. Each treatment lasted for 5 consecutive days, and a period of 30 days was allowed for washout after previous treatments. Both groups were similar in physical characteristics and on season training. Body composition (body weight, % body fat, lean body mass, total body water, body mass index), blood creatinine, vertical jumping tests, anaerobic, and capacity, and swimming performance (take-off force at 50-m, speed at 50-m.) were measured before and 5 days after placebo or creatine administration at both doses in each group. There were no effect of creatine at both doses on most parameters of body composition, except total body water which was increased after creatine supplementation at high dose. Blood creatinine concentrations were not changed after 5 days creatine supplementation. However, anaerobic capacity was increased whereas fatigue index was decreased after high dose creatine administration. On the other hand, swimming speed at 50-m was significantly increased by both low and high dose of creatine supplementation.

This study support a positive beneficial effect creatine supplementation, especially at high dose, on swimming performance of Thai collegiate swimmers. Such treatment appears to have no undesirable effect on the body weight nor the kidney function.

**SC 4:** Local Anesthetic Effect of Pellitorine, An Active Compound Isolated from CHA-PLU Fruit (Piper sermentosum Roxb.)

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The present study aimed to investigate local anesthetic effect of a semi-purified compound, containing mainly pellitorine, isolated from Piper sarmentosum Roxb. on isolated frog sciatic nerves. In addition, in vivo effect of the test compound was further investigated in mouse tail flick test and twitch-response test in guinea pig’s skin.

The test compound was dissolved in 0.1 M hydroxypropyl-β-cyclodextrin in Ringer’s solution and being applied onto frog sciatic nerve. It was found that pellitorine in the concentration of 10 - 25 mM exerted a concentration-dependent manner and reversible blockade of supramaximally electrical-evoked action potential of isolated frog sciatic nerves. Complete block
electrical-evoked action potential exerted by 25 mM of pellitorine and 30 mM of lidocaine was achieved at 73.75 ± 2.26 min and 7.50 ± 0.94 min respectively. Further investigation in mouse tail flick test, using analgesia meter, revealed in vivo local anesthetic effect of test substances which was injected into mouse’s tail base. The EC$_{50}$ of pellitorine and lidocaine in this model were found to be 16 and 12 mM, respectively. This observation was subsequently confirmed by twitch response in which the test substances were infiltrated intradermally on the back of guinea pig and pricking was used to probe for response to pain. Apparently, pellitorine (3 - 14 mM) as well as lidocaine (4 – 16 mM), elicited analgesic effect with the duration of sensory block of about 30 – 50 min.

Based on the results obtained it can be concluded that pellitorine possesses in vitro as well as in vivo local anesthetic effects. The finding that the degrees of conduction block elicited by pellitorine or lidocaine on frog sciatic nerve were markedly increased in sodium deficient Ringer’s solution, suggesting the similar mode of action of theses two compounds. Further investigation on the effect of pellitorine on sodium channel is required to elucidate the mechanism of local anesthetic observed. In addition toxicity which may arise should also be evaluated and that may lead to a discovery of a local anesthetic from natural source.

**SC 5:** Transition of LAT1 to LAT2 in Kidney Development
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System L amino acid transporters are heterodimeric, composing of a heavy chain, 4F2hc with a light chain, either LAT1 or LAT2. Recently, LAT1 is shown to be highly expressed in fast growing, including cancerous, cells. Furthermore, some fetal but not adult organ such as liver expressed LAT1. In contrast, LAT2 is highly expressed in normal epithelial tissues especially kidney and intestine. We therefore hypothesized that during fast growing organogenesis LAT1 may preferentially be expressed. To substantiate the above idea, the changes in the expression of LAT1, LAT2 and 4F2hc were studied in the mouse kidney from embryonic to adult stage.

The mRNA expression levels of these genes were quantitatively analyzed by Taqman PCR. We clearly demonstrated that the LAT1 is highly expressed in embryonic day 15 (E15) kidney and gradually declined with development and became very low after weaning, 3 weeks after birth. In contrast, the low expressions of LAT2 during early stage increased with the development from the E15 kidney till birth and reached the highest level during wean. LAT2 expression was slowly declined to reach a steady level of half the peak value at 7 weeks after birth. Thus the expressions of LAT1 and LAT2 during kidney development from E15 to wean were regulated in the opposite direction. The expression levels of LAT1 and LAT2 protein from western blot analysis showed the similar pattern of mRNA expression. We concluded that LAT1 is highly expressed in the E15 kidney and may be important for amino acid transport only in highly proliferating cells during organogenesis. During development, LAT1 expression is gradually suppressed, whereas LAT2 and 4F2hc are increasingly expressed. The expression of these transport proteins may inversely be regulated possibly within the same tubular cell of the kidney.

**SC 6:** Lipid Peroxidation in Renal Ischemic Reperfusion: Effect of Angiotensin Inhibition
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Objectives: To investigate the role of angiotensin on lipid peroxidation (LPO) and nephropathy in renal ischemic reperfusion (IR).

Background: During IR, both reactive oxygen species (ROS) and angiotensin II (ANG II) are increased and play an important role in renal injury. ANG II increases ROS (O$_2^-$) through activating membrane associated NADH/NADPH oxidase activity. ROS can produce cellular injury by attacking membranes through the peroxidation of polyunsaturated fatty acids. LPO of mitochondrial, lysosomal and plasma membrane could alter both membrane structure and function.

Materials and methods: Male Wistar rats were subjected to 15-, 30-, 45- or 60- minute left renal artery occlusion (I). In 30-minute I group, the additional animals were followed by reperfusion (R) for 1 day in which were treated with water or angiotensin converting enzyme inhibitor (ACEI; enalapril 5 mg/kg/d) or angiotensin receptor type 1 antagonist (ARA; losartan 10 mg/kg/d). Renal tissue malondialdehyde (MDA), a secondary product of oxidative stress formed during LPO was measured. Renal pathology also was determined.

Results: Renal tissue MDA levels were progressively increased with increasing time of ischemia and was maximal at 30 minute (p<0.01). One day after reperfusion, the MDA level was still high (p<0.01) as compared to control. Both ACEI and ARA could attenuate the heightened MDA levels (p<0.01). Histological changes in 30-minute I group were slightly tubular dilatation and congestion. The progression of renal pathology was observed after 1 day of reperfusion. IR-induced nephropathy was ameliorated by ACEI or ARA administration.

Conclusion: Inhibition of angiotensin has a protective effect on lipid peroxidation and nephropathy during IR condition.

SC 7: Expression of Renal eNOS Protein during Unilateral Ureteral Obstruction: Role of Angiotensin System

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Objectives: To investigate the role of angiotensin on renal nitric oxide synthase (NOS) protein expression, nitric oxide (NO) production and nephropathy in unilateral ureteral obstruction (UUO).

Background: Angiotensin and nitric oxide play critical roles during UUO. No clear studies have demonstrated the role of angiotensin on renal eNOS protein expression in this condition.

Materials and methods: Male Wistar rats were divided into two main groups: sham (S) and UUO. In UUO group, there were 3 subgroups treated with: 1) water, 2) angiotensin II receptor antagonist (ARA, losartan: 500 mg/L), and 3) angiotensin converting enzyme inhibitor (ACEI, enalapril: 200 mg/L) for 1 or 7 days. Renal eNOS protein expression and nephropathy were examined. NO production (nitrite) was determined.

Results: UUO for 1 or 7 days increased renal eNOS protein expression. Treatment with ACEI or ARA slightly reduced the expression caused by UUO in 1-day group. In 7-day animals, the expression was maintained in cortex and was further increased in medulla after ACEI or ARA administration. UUO enhanced serum nitrate concentration (p< 0.05). ACEI and ARA could normalize the heightened nitrite level induced by UUO to be near sham. The 1-day UUO kidney showed a mild tubular dilatation and some cell infiltration. Both ACEI and ARA could attenuate structural alterations. The 7-day UUO rats demonstrated progressively morphological changes. ACEI had more effectiveness in reduction of tissue destruction than those of ARA.
Conclusion: Angiotensin could attenuate renal eNOS protein expression in 1-day UUO group but not in 7-day UUO animals. Inhibition of angiotensin ameliorates increased NO production and nephropathy induced by UUO.

SC 8: Genetic Polymorphism of the CYP2A6 Gene and Smoking Behavior in The Northern Thai Subjects
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Human cytochrome p450 (CYP) 2A6 plays an important role in the metabolic inactivation of nicotine which is responsible for smoking addiction. Genetic polymorphisms in the CYP2A6 gene have been shown to have large interindividual and interethnic variability in levels of expression and activity. Therefore, this study is aim to investigate the CYP2A6 allele frequencies and to elucidate whether there is the relation between the CYP2A6 polymorphisms and smoking behavior in the Northern Thai subjects. The CYP2A6 genetic variations were determined by a PCR-based method coupled with RFLP and allele-specific PCR analysis in 118 nonsmoke healthy controls and 123 dependent smokers (defined by drug dependence assessment with DSM-IV for nicotine and Fagerstrom Test of Nicotine Dependence (FTND) criteria). The frequencies of CYP2A6*1A, CYP2A6*1B (designed as wild-type), CYP2A6*4C (whole deletion), CYP2A6*7 (SNP in exon 9: T1412C) and CYP2A6*9 (SNP in TATA box: T-48G) variants were 37.71%, 33.47%, 5.51%, 6.36%, and 16.67%, respectively, among controls compared with 30.08%, 34.15%, 9.35%, 9.76% and 16.67%, respectively, among smokers (χ²; p>0.05). Subjects who carried homozygous mutant alleles (13.00%) tended to smoke fewer cigarettes (10.06±4.78 vs 11.25±6.96 cigarettes per day; ANOVA p>0.05) than those who genotyped as homozygous wild type (41.50%), although there was no statistically significant correlation between the CYP2A6 variants and number of cigarettes smoked per day because of the small number of samples. These results demonstrated that the CYP2A6 genetic polymorphisms do exist in various frequencies and may alter the smoking behavior in the Northern Thai populations.

SC 9: Effect of Antioxidant Quercetin Supplementation in Phenylhydrazine-Induced Hemolysis in Rats
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Objective: To examine whether quercetin can reduced oxidative damage in phenylhydrazine (PHZ)-induced hemolysis in rats.

Background: Quercetin is a potent antioxidant and one of the most abundant flavonoids in the human diet. Free radical plays a major role in the pathogenesis of phenylhydrazine (PHZ)-induced hemolytic anemia.

Materials and methods: Male Sprague-Dawley rats were orally administered with quercetin (50 mg/kg/day) or deionized water as controls for 6 days. On the fourth day of treatment, all of studied animals were induced hemolytic anemia by a single injection of PHZ (125 mg/kg i.p.). After PHZ administration for 48 hours, animals were anesthetized for measurements of mean arterial blood pressure (MAP), hindlimb blood flow (HBF) and hindlimb vascular resistance (HVR). In addition, glutathione (GSH), malondialdehyde (MDA) and nitric oxide metabolites (NOx) were assayed.
Results: The results demonstrated that quercetin could elevate the cellular antioxidant system by increased the intracellular concentration of GSH, whereas plasma level of MDA and NOx were decreased in anemic rats (p<0.05). In view of hemodynamic responses, MAP and HVR of the anemic rats treated with quercetin were increased while HBF was decreased when compared to the controls (P<0.05).

Conclusion: Quercetin is an effective antioxidant to alleviate the consequent effect of PHZ and improved circulatory function in anemic rats, and suggest a further application in diseases accompanied by free radical damage.

**SC 10**: Relationship Between Iris Blood-Flow Perfusion and Leukocyte Adhesion in Diabetic Rats: Role of Vitamin C

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Objective: To study the effects of vitamin C on the relationship between leukocyte adhesion and iris blood-flow perfusion in diabetic rats, the animal model of streptozotocin (STZ)-induced diabetic rats was used.

Materials and Methods: Male Wistar Furth rats weighing 200-250g were divided randomly into three groups of control (CON), streptozotocin (STZ; a single intravenous injection of STZ; 55 mg/kg BW) and STZ-supplementation with vitamin C (STZ-Vit C; free access to drinking water (1 g/L of ascorbic acid (Sigma, Chemical Co., USA)).

On the day of experiment, body weight (BW), blood glucose (BG), glycosylated hemoglobin (HbA₁c), plasma vitamin C, malondialdehyde (MDA), iris blood-flow perfusion, and leukocyte-endothelial cell interaction were evaluated at 8, 12, 16, 24 and 36 weeks after injection of STZ for all groups.

Results: The results showed that all groups of STZ had the significantly increase in BG, HbA₁c, MDA, and in number of leukocyte adhesion, but decrease in BW, plasma vitamin C, and iris blood-flow perfusion as compared to their age-match controls. However, 36-week STZ-Vit C was significantly decreased in BG, HbA₁c and MDA as compared to STZ. Moreover, 24- and 36-week STZ-Vit C were significantly increase iris blood-flow perfusion but decrease leukocyte adhesion as compared to STZ. The relationship between iris blood-flow perfusion and leukocyte adhesion were able to represent by the linear equations of \( y = -0.447x + 32.80 \), \( r = -0.317 \) (p < 0.034) and of \( y = -1.862x+47.10 \), \( r = -0.517 \) (p < 0.001), for STZ-rat and STZ-Vit C, respectively.

Conclusion: The leukocyte vaso-occluded was not a simply major cause of abnormalities of such iris blood-flow perfusion. And not only vitamin C supplementation can prevent the diabetic-induced endothelial impairment but also it might be a great therapeutic agent for preventing abnormality in diabetic eyes.

**SC 11**: Effect of Vitamin C on Endothelial Nitric Oxide Synthase Expression in Systemic and Pulmonary Circulation of Streptozotocin-Induced Diabetic Rats: Quantitative Comparison Using Image Digital Analysis

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Background: Several studies have indicated that increased reactive oxygen species (ROS) might be the possible key factors in diabetic endothelial dysfunction. ROS could decrease nitric oxide (NO) either via scavenger NO molecules directly or by damaging eNOS-protein.

Objective: To investigated whether the supplementation of the antioxidant, vitamin C, in streptozotocin-induced diabetic rats (DM) will be able to prevent such effects of ROS on eNOS expression produced in heart and lung vascular tissue or not.

Materials and Methods: eNOS levels was studied in heart and lung extracts at 12 and 24 weeks of control rats (CON), streptozotocin (STZ)-induced diabetic rats (DM) and diabetic rats supplementation with vitamin C (DM+Vit. C). The single intravenous injection of 50 mg/kg BW STZ was used. Western blot analysis using monoclonal antibody against rat eNOS protein (140 kDa) was performed. From images of eNOS bands on each film, the number of pixels in each digital file were counted and calibrated for eNOS-protein content. The mean values of image analyzed amount of eNOS-protein contents were calculated and expressed as a percentage of total protein content.

Results: In DM hearts, eNOS proteins were significantly decreased as compared to age-matched CON hearts. Interestingly, in DM+Vit. C hearts, there was a significant increase eNOS protein levels compared to age-matched DM. On the other hand, it was found that the eNOS protein levels in DM lungs were significantly increased, compared to CON lungs. However, DM+Vit. C lungs had shown their eNOS protein levels higher than CON lungs.

Conclusion: Our results demonstrated that the reduction of eNOS protein progressive with the diabetic stage was only found in the heart, not in the lung. Interestingly, the supplementation of vitamin C had effect in diabetic-induced endothelial impaired, especially to prevent eNOS-protein damage.
THE 33rd ANNUAL ACADEMIC MEETING OF THE PHYSIOLOGICAL SOCIETY OF THAILAND

Abstract: poster session
**P 1: Maturity of Pericytes in Cerebral Neocapillaries Induced by Growth Factors: A Confocal Laser Microscopic Study**

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From the study of H. Niimi and co-worker (1,2), cerebral angiogenesis was induced in mice by implanting a sandwich system of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) gel and nylon-mesh over the exposed cortex. On 28th day after incubation of these growth factors, the results were demonstrated that neocapillaries induced by PDGF was dilated in response to acetylcholine (ACh), which was topically applied on the neocapillaries, while the bFGF group was not. Therefore, in the present study the maturity of pericytes in cerebral neocapillaries was examined using an immunohistochemical staining technique. On 28th day after incubation, a small volume of cerebral tissue with nylon mesh was isolated and stained with tetramethyl rhodamine isothiocyanate (TRITC)-labeled Griffonia simplicifolia (GS)-lectin. Confocal laser microscopic system was used for observation the cerebral neocapillaries on the upper surface of the nylon mesh and evaluation the maturity of pericytes stained with NG2 based on the fluorescence immunohistological image. The immunohistochemical study for NG2 expression demonstrated the appearance of pericytes in PDGF-induced neocapillaries. These pericytes may play a role in the vasodilatory response to ACh and control of blood flow in the cerebral neocapillaries.

**P 2: Mechanisms of VR-3623-Induced Apoptosis in Human Lung Cancer LU-1 Cells**

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At present, it has been demonstrated that most anticancer drugs induce cell death via apoptosis. VR-3623 (5,3', dihydroxy-3,6,7,8,4'-pentamethoxyflavone), purified from a Thai tropical plant, Gardenia obtusifolia, has been shown to inhibit cell growth and induce apoptosis in some human cancer cell lines which have been studied. The aim of the present study was to investigate the mechanisms of flavone-induced apoptosis in human lung cancer LU-1 cells. To elucidate these mechanisms, LU-1 cells were treated with 30 M of VR -3623 for 48 hours. Cells undergoing apoptosis were detected by 4',6-Diamino-2-phenylindole (DAPI) staining and agarose gel electrophoresis for morphological and biochemical changes, respectively. Further, the release of mitochondrial cytochrome C to the cytosol and activation of caspase-3 during apoptosis were examined by immunoblot analysis. Furthermore, the caspase-3 like activity in the VR-3623-induced apoptosis was also measured by a fluorogenic assay. The results showed that the number of apoptotic cells characterized by condensed and fragmented nuclei increased with time. Apoptotic cells could be observed since 3 hours after incubation and reached the maximal value approximately 68 % at 24 hours. Thereafter, the most cells underwent DNA fragmentation. This effect was demonstrated by the appearance of “DNA ladder” in agarose gel at 48 hours. VR-3623 treatment resulted in a
3.3-fold increase of cytochrome c release from mitochondria at 12 hours. This was the concomitant of the activation of caspase-3 as revealed by decreased proform of caspase-3 and subsequently increased activity of caspase-3 like enzyme since 24 hours. Moreover, ten micromole of caspase-3 inhibitor (DEVD-CHO) completely inhibited the caspase-3 like activity. Taken together, it is concluded that apoptosis mediated by VR-3623 in LU-1 cells is, at least in part, caspase-3-dependent via the release of cytochrome C.

**P 3:** Metabolic Responses to Exercise After Oral and Intravenous Carbohydrate Loads in Healthy Men and Women
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Objectives: This study aimed to investigate gender differences in i) pancreatic insulin secretory (â-cell sensitivity) and insulin sensitivity responses to an intravenous carbohydrate (CHO) load and (ii) metabolic responses to exercise after intravenous and oral CHO loads.

Background: During exercise performed in the fasting state, women oxidise more lipid and less carbohydrate (CHO) than men, resulting in differential substrate utilisation. This gender difference in energy metabolism is likely to be enhanced if exercise is to be performed in the postprandial state, ie at a time of relatively high circulating glucose and insulin concentrations. Furthermore, a gender difference in â-cell and insulin sensitivity may further affect the metabolic responses to moderate exercise after a CHO meal. However, it is unclear whether there is a gender difference in â-cell sensitivity and whole body insulin sensitivity.

Methods: Untrained healthy seven men and seven women performed two trials. In one trial they cycled for 60 min at 50% VO_{2max} 60 min after ingestion of a carbohydrate-rich meal (ME trial). In the other trial, subjects were infused with 20% dextrose solution until blood glucose concentration reached ~ 8 mmol/l for 60 min (INF trial), then this infusion rate was maintained constant during the following 60 min while exercising at 50% VO_{2max}.

Results: There were no gender effects on â-cell (26.9 ± 6.2 and 26.5 ± 4.6 µU/ml for men and women, respectively) and whole body insulin sensitivity (25.8 ± 4.0 and 22.5 ± 4.8 mg/(kg FFM·min) per µU/ml·100 for men and women, respectively). This may explain the similar glycemic, substrate oxidation and other metabolic, except for plasma epinephrine concentration which was lower (P < 0.01) in men than women, responses to exercise after intravenous and oral CHO loads.

Conclusion: There were no gender differences in â-cell sensitivity and insulin sensitivity responses to an intravenous CHO load and metabolic responses to exercise after intravenous and oral CHO loads.

**P 4:** Acute and Chronic Hypotensive Effects of Crude Leaf Extract of Nelumbo nucifera Gaertn.
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The leaves of Nelumbo nucifera Gaertn. (N. nucifera) have been used in traditional medicine for antihypertensive purpose. Scientific evidence regarding its hypotensive activity has not yet been documented. The present study was aimed at investigating chronic and acute effects of crude leaf extract of N. nucifera (EN) on blood pressure in renal hypertensive and normotensive rats.

Hypertension was produced by constriction of left renal artery in male Sprague-Dawley rats. Systolic blood pressure (SBP) was measured weekly by a tail-cuff plethysmography. After being established in hypertensive state, rats were divided into 4 groups: non-treated, and treated with EN 100, 400, and 800 mg/kg BW. In the treated groups, rats had been fed with EN daily for 3 weeks. The results showed that the SBP of control and EN 100 mg/kg BW treated group slightly increased by 2-6% from initial values during the period of observation. In comparison, in the last two weeks of treatment EN 400 and 800 mg/kg BW significantly lowered SBP by 8-9% and 4-10% from initial values respectively (P<0.05 vs control). The heart rate (HR) was not significantly different between groups over the test period.

The hypotensive activity of EN was confirmed in normotensive rats by acute intravenous administration at doses of 0.1 and 1 mg/kg BW. Arterial blood pressure and HR were monitored from carotid artery. It was found that EN caused a rapid and significant decrease in systolic and diastolic blood pressure (DBP) in a dose-dependent manner (0.1 mg/kg BW: SBP 82.15±1.86%, DBP 70.38±2.54% of initial BP; 1 mg/kg BW: SBP 72.86±2.27%, DBP 55.37±3.89% of initial BP; P<0.05). The HR showed significant increases after EN administration (P<0.05 vs initial HR), indicating reflex compensation for the decrease in BP. The hypotensive effects of EN 1 mg/kg BW were completely blocked by atenolol (β-adrenergic blocker) 5 mg/kg BW.

In conclusion, EN can exert both chronic and acute hypotensive effects, which may be mediated via β-adrenergic receptors in blood vessels.

P5: The effect of a Thai herbal medicine on cerebral activity detected by functional magnetic resonance imaging in the rat

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Background: Thunbergia laurifolia Linn (TH) is a herbal medicines used as an antidote for several poisonous agents in Thai traditional medicine. This plant has also been used extensively as component of mixture of crude extracts to treat patients with drug addiction (1) but there are no clinical trials on the use of TH in the treatment of drug addiction. Recently, we demonstrated that amphetamine and TH significantly increased potassium-stimulated dopamine release from rat striatal slices when compared to potassium-stimulated alone. TH potentiated the effect of amphetamine on potassium-stimulated dopamine release when compared to amphetamine alone. The results indicate that TH may stimulate dopamine release in the same manner as amphetamine (2).
Objective: This study aims to test the effect TH on cerebral activity detected by functional magnetic resonance imaging (fMRI) in the rat.

Material and method: fMRI was performed on a 2.35T Bruker Biospec Advance MR System. Consecutive single slice functional imaging over the whole of the rat brain was performed to investigate the changes in signal intensity at various parts of the brains induced by TH (200 mg/kg) or vehicle action.

Results: The fMRI study demonstrated that TH increases % signal intensity at various areas in the brains such as amygdaloid nuclei, parietal cortex, frontal cortex, nucleus accumbens, caudate putamen and hippocampus.

Conclusion: The result was the first to indicate that TH activates various parts of brains responsible for reward and locomotor similar to the effect of amphetamine and cocaine previously reported (3). Further studies are required to investigate the value of the plant for the treatment of drug addiction.

P 6: Distribution of Neuronal Nitric Oxide Synthase Isoform in Bovine Hippocampus
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Nitric Oxide (NO) is a diffusible regulatory molecule involved in a wide range of physiological and pathological events. The intracellular NO production is catalyzed by Nitric Oxide Synthase (NOS) enzymes. The aim of this study was to investigated the distribution of neuronal isoform of NOS (nNOS) enzyme in the bovine hippocampus sections by using immunohistochemical staining. The result showed that there were brown pigments from immunohistochemical reaction in cytoplasm of pyramidal neurons located in area CA1 and CA3 of bovine hippocampus sections. The distribution of nNOS in the reactions was different from area to area. The most intent of nNOS immunoreactivity was in CA1 and CA3 whereas the lowest immunoreactivity was in mid-hippocampus area. Our finding might imply that the quantity of nNOS in pyramidal neurons was various in each part of hippocampus.

P 7: Screening the Neuroprotective Effect of Arcangelisia flava on Various Areas of Cerebral Cortex
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Background: Recently, alcohol problems has increased considerably in Thailand. Alcohol can induce neuronal damage in various areas of brain via the increase in free radical levels. Plant containing antioxidant activity such as Arcangelisia flava which is widely used in Indo-China region as antidiabetic drug may possibly benefit for the protection of brain from ethanol.

Objective: To determine the protective effect of ethanolic extract of Arcangelisia flava on neurotoxicity induced by ethanol in various areas of cerebral cortex.
Method: The ethanolic extract of bark of Arcangelisia flava at various doses ranging from 1, 5, 10 and 20 mg/kg BW was administered to the rats via intragastric route for 14 days before the administration of ethanol at dose of 2 g/kg BW for 2 weeks. After the last dose of treatment, they were determined the density of neurons in various areas of cerebral cortex using histochemical techniques.

Results: Ethanol reduced neuronal density in various areas of cerebral cortex. However, the ethanolic extract of Arcangelisia flava at dosage range used in this study did not show neuroprotective effect against ethanol.

Conclusion: This study shows that ethanolic extraction of Arcangelisia flava can not be used as neuroprotective agent against ethanol.

P 8: Anticytotoxicity Effect Induced by Ethanol of Morus alba in The Rat Brain
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Background: Morus alba or mulberry tree is an interesting plant and it is also an economic plant in Northeastern of Thailand that tree has many medicinal values and were used as medicinal plant for along time. Its extract possesses an antioxidant activity. Chronic alcohol consumption results in brain damage and neurological dysfunction in both humans and animals partly via inducing oxidative stress. To date, whether pretreatment with mulberry tree extract protect ethanol induced brain injury has not been established.

Objective: The present study, investigates the protective effect of the mulberry tree extract on the cytotoxicity of ethanol in the brain.

Methods: Young adult male Spargue-Dawley rats, 8 weeks old were divided randomly into 3 groups: control, ethanol and mulberry plus ethanol treated group. In the first 14 days, rats in control and ethanol treated groups were given 0.9% NSS via orally, while those in mulberry plus ethanol treated groups were orally given mulberry extract at various doses (0.5, 1, 2, 5 and 10 mg/kgBW). The last 14 days, the control group was subcutaneously injected with 0.9% NSS whereas the ethanol and mulberry plus ethanol treated groups were subcutaneously injected with ethanol 20 mg/kgBW. At the end of treatment period all animals were sacrificed, then their brain were removed. Frozen brain serial section 40µm thick was obtained and the survival of neurons was investigated using Nissyl histochemistry technique.

Results: Mulberry tea extract at dose 1 mg/kgBW showed significant difference from control group in frontal, parietal, striatum, dentate gyrus and CA1 (p<0.05).

Conclusion: The present data indicate that mulberry tree would prevent the cytotoxicity induced by ethanol.

P 9: The Study of the anxiolytic actions of Noni juice (Morinda citrifolia L. Rubiaceae) in the male Wistar rats
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Noni (Morinda citrifolia L. Rubiaceae) juice, a type of health-promoting beverages, was reported to have a broad range of therapeutic effects, including sedation, analgesic and anti-inflammatory. However, the scientific reports on its effects have not been clarified. The present study was interested in the anxiolytic action of noni juice or actions on the central nervous system. Elevated plus maze (EPM) was selected for testing the anxiolytic-like behavior in male Wistar rats. Body weight, food consumption, and blood chemistry specific for liver and kidney profiles were also monitored. Rats were fed orally with noni juices, which were Tahitian noni juice (TNJ) or Siam noni juice (SNJ), for 15 or 30 days. There was no different in body weight, daily weight gain, daily food intake, or the blood chemistries between treatments after noni juices given for 15 or 30 days. For the anxiety-like behavior, when noni juices were fed for 15 days, both SNJ- and TNJ-treated rats spent longer time on opened-arm than control group similarly to diazepam-treated rats. SNJ-treated rats, the percentage of entry into opened-arm increased while TNJ- and diazepam-treated rats were not different from control. The data indicated that noni juices contained an anxiolytic activity. The prolonged feeding for 30 days had similar effects to 15 days; however, the grooming activity disappeared in 30 days TNJ- and SNJ-treated groups. This suggested that the prolonged feeding could further reduce anxiety-related behavior in rats.

P 10: P53 Codon 72 Polymorphism and Risk Factors of Squamous Intraepithelial Cervical Lesions in Patients at Srinagarind Hospital
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Background: Polymorphism of the tumor suppressor gene p53 at codon 72 may increase the risk of cancer. It is unclear whether persons carrying the arginine vs. proline form are more susceptible to squamous intraepithelial cervical lesions (SIL). Reproductive factors, sexual behavior and smoking are associated with development of SIL albeit the temporal relation remains undefined.

Objectives: To determine the association of squamous intraepithelial cervical lesions (SIL) and p53 codon 72 polymorphism, and of SIL and the risk factors.

Patients and Methods: The KKU Ethics Committee approved the study and written, informed consent was obtained from the subjects: 45 patients with SIL at Srinagarind Hospital, and 111 healthy controls. DNA was extracted from peripheral blood and polymorphism of p53 was identified using PCR-RFLP. Risk factors were discovered during interviews. The association between SIL, the risk factors, and polymorphism of p53, were analyzed using STATA software.

Results: The difference in the distribution of p53 genotypes among patients and controls was not significant. Heterozygous, Pro-homozygous and Arg-homozygous in the SIL vs. control group were 67, 18 and 16 vs. 62, 18 and 19 percent, respectively. Homozygosity for Arg/Arg was not associated with any increased risk for squamous intraepithelial cervical lesions (OR 0.79; 95%CI = 0.20-3.04). Logistic regression analysis found no
association with SIL. Age at menarche, number of parity, age at first intercourse, number of sexual partners, and history of STDs, did not significantly affect SIL (p value $\geq 0.5$).

Conclusion: Polymorphism of p53 at codon 72 may not be a risk for squamous intraepithelial cervical lesions in women in Northeast Thailand. Sexual behaviors and lifestyles did not increase the risk.

**P 11**: A Study of Tumor Neocapillary Density Using Intravital Fluorescence Videomicroscopy and Dorsal Skin-fold Chamber Implanted Nude Mice Model

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Objective: To investigate the development and remodeling of the tumor neogenesis occurred in dorsal skin-fold chamber implanted nude mice, the intravital fluorescence videomicroscopy was used.

Methods: Male BALB/c nude mice weighing 20-25g were used. After the implantation of a dorsal skin-fold chamber, the Hepatocellular carcinoma cells (HepG2) (30 $\mu$l of 2 x $10^6$ cells) were then inoculated onto the upper layer of the skin within the chamber (HepG2-group, n=12). The group of sham control (Sham-group, n=12) were received the normal saline instead. The intravital videomicroscopic measurements were performed to monitor the tumor neocapillary on days 7 and 14 post-inoculation by using 0.1 ml of 0.5% rhodamine B isothiocyanate-labeled dextran (RITC-dextran). Values of tumor neocapillary density, diameters, and vasculature were evaluated in correlation with the tumor area by using digital image analysis (Global Lab II Software, Datatranslation Co., USA).

Results: The tumor cell in the chamber appeared within 24 hours after the inoculation as which confirmed by H&E examination under the pathologist reported.

The results of intravital fluorescence videomicroscopy with the image analysis, it was demonstrated that neocapillary density of HepG2-group were significantly increased on day 14 as compared to the aged-matched Sham-group (p<0.05). In addition the increase of neocapillary density was correlated with the tumor growth, as well as the tissue perfusion.

Conclusions: Dorsal skin-fold chamber technique together with intravital videomicroscopy and the application of digital image analysis will allow us to online-plus-in vivo monitoring the tumor neocapillary. Therefore, it might be used as an in vivo model for evaluating any anti-angiogenic agents in the future.

**P 12**: Effect of *Aloe vera* on Leukocyte Adhesion and Cytokine-Levels in Burn-Wounded Rats Model

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Objective: To study the effects of Aloe vera on leukocyte-endothelial cell interaction and on TNF-α and IL-6 levels in burn-wounded rat model.

Materials and Methods: Seventy-two male Wistar Furth rats were equally divided into 4 groups of control (CON), untreated burn-wound rats (BURN), normal-saline-treated burn-wound rats (BURN-NSS) and Aloe vera daily-treated burn-wound rats (BURN-aloe). Dorsal skin-fold chamber preparation and intravital fluorescence microscopic technique were performed to examine leukocyte adhesion on postcapillary venules on day 3, 7 and 14 postburn. ELISA techniques were performed to examine serum TNF-α and IL-6 levels. Besides, at the end of each experiment the specimen from the burn area were collected for further histological examination using H&E.

Results: The amount of leukocyte adhesion was significantly reduced in BURN-aloe group as compared to BURN group. Levels of TNF-α and IL-6 were also decreased significantly compared to BURN at all 3 monitored time points. Furthermore, the H&E examination demonstrated that epithelialization was fully developed on day 14 in aloe-treated group.

Conclusion: Aloe vera could inhibit the inflammatory process characterized by decreasing leukocyte adhesion, as well as TNF-α and IL-6 levels. Besides, wound healing acceleration described by epithelialization and wounded areas was also observed in our study.

P 13: Anti-Inflammatory Activity of Mallotus repandus

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Objective: To demonstrate the anti-inflammatory activity of Mallotus repandus extract in acute inflammatory models

Background: M. repandus, family Euphorbiaceae, is a tropical shrub up to 3 m tall. It has been used in folklore medicine in many countries in Southeast Asia, including Thailand, and used as an insecticide, anti-allergy and as a remedy for fever, rheumatoid arthritis, snakebite, hepatitis, and liver cirrhosis. In Thailand, it was one of the most popular plants that used in the treatment of muscle and joint pain.

Materials and Methods: Anti-inflammatory activity of the methanol extract from the stem of M. repandus was studied by using both in vitro and in vivo inflammatory models. The eicosanoid production by ionophore stimulated rat peritoneal leukocytes and rat hind paw edema models were used as an inflammatory model in in vitro and in vivo study, respectively.

Results: The methanol extract from the stem of M. repandus inhibited the cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism in rat peritoneal leukocytes stimulated with calcium ionophore A 23187 in a dose dependent manner. In the rat paw model test, the extract showed significant anti-inflammatory effect when given intraperitoneally. Noticed that, the anti-inflammatory effect of the extract with the dose of 500 mg/kg was comparable to oral aspirin (300mg/kg).

Conclusion: With the preliminary study, the methanol extract from the stem of M. repandus possessed significant anti-inflammatory activity in acute inflammatory models.
P 14: The Effect of Combretum decandrum Roxb. on Glucose Tolerance of Hyperinsulinemic Rats
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Objective: This study had an aim to examine the effect of Combretum decandrum (CD) extract on the glucose tolerance of type 2 diabetic rat model.
Background: Hyperinsulinemia leading to insulin resistance is the major factor in the pathogenesis of type 2 diabetes mellitus. Interestingly, the result from our previous study indicated that CD extract possessed hypoglycemic effect in type 1 diabetic rat model and enhanced glucose uptake of fat cells of normal rats. Therefore, this study examined the effect of CD extract on the glucose tolerance of hyperinsulinemic rats.
Materials and Methods: The long-acting insulin at 2 IU/KgBW was injected subcutaneously three times daily into Male Wistar rats for 15 consecutive days. The oral glucose tolerance test was performed on the day before and 3 days after the administration of ethanolic extract of CD.
Results: The increase in blood glucose of hyperinsulinemic rats, at 120 min. after oral administration of glucose (3 g/KgBW), was significantly (P<0.05) higher than that of normal rats (42.45±5.60 and 3.04±5.77 % respectively). After receiving CD extract at doses of 0.5 and 0.75 g/KgBW, the percent increases in blood glucose of hyperinsulinemic rats was significantly lower as compared to before receiving CD extract (2.86±9.77 and 24.86±5.90% respectively).
Conclusion: The CD extract at doses of 0.5 and 0.75 g/KgBW can improve the glucose tolerance of hyperinsulinemic rats.

P 15: Evaluation of Anti-hyperglycemic Action of Steviol
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Steviol (SVO) is a metabolite of stevioside which is a major component of sweet herb stevia rebaudiana Bertoni. SVO has been found to potentiate insulin release from isolated islet of Langerhans. It is presumably that SVO decrease plasma glucose. Therefore, the present study was carried out to clarify the glycemic action of SVO in both normoglycemia and hyperglycemia. Hyperglycemia was produced by either streptozotocin to damage β-cells (STZ-induced hyperglycemia) or intravenous infusion of glucose (intravenous glucose tolerance test, IVGTT). For IVGTT, 3 mg/kgBW was intravenous injection and followed by glucose loading (2 g/kgBW). To evaluate the glycemic action of SVO in cells STZ-induced hyperglycemia, 6 periods of 30-min were established including of 2 control periods, 2 SVO periods (3 mg/kgBW/hr) and 2 recovery periods. The results indicate that SVO reduced area under curve of glucose in IVGTT from 1377.53 ± 26.46 to 1168.21 ± 54.99 mM x120 min (P < 0.01). Area under curve of insulin was increased from 496.79 ± 82.34 to 718.73 ± 27.94 ng/ml x120 min (P
No significant change of plasma glucose level in STZ-induced hyperglycemia either treated with or without SVO. It can be concluded that SVO improves hyperglycemia induced by glucose loading whereas no significant effect on plasma glucose in hyperglycemic rats with β-cells damaged by STZ. The mechanism of its hypoglycemic action is possibly correlated to glucose-induced insulin release.

P 16: Studies on Hypoglycemic Effect of Momordica charantia Linn. and Arcangelisia flava Merr. in Normoglycemic and Hyperglycemic rats
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Fresh juice of Momordica charantia Linn. (MC) has been found to reduce blood glucose level in normal rats. However, no experimental evidence was undertaken to compare the glycemic effect of its various parts. In addition, no information to support the glycemic action of Arcangelisia flava Merr. (AF). Present study aimed to clarify the glycemic action of both MC and AF in normoglycemia and hyperglycemia. Male Wistar rats were induced to be hyperglycemia by either oral glucose loading or streptozotocin (STZ) injection. Three preparations of fresh MC were performed including pulp juice without seed and cuticle (juice A), pulp juice without seed (juice B) and whole fruit juice. AF was extracted by 95% ethanol. After overnight fasted, each rat was fed by either 10 ml/KgBW of MC or tolbutamide (90 mg/kgBW) or aqueous extract of AF (0.25, 0.5, 1 g/kg BW). Oral glucose tolerance (OGTT) was also undertaken after either MC or AF feeding. The results indicate that BG was significantly decreased at 4 hrs after juice A feeding (~16%) whereas it decreased both 2 and 4 hrs after juice B feeding (~15%) in normal rats. The maximum hypoglycemic activity was observed in normal rats fed with whole MC juice (~24%) which was comparable to tolbutamide. In STZ-induced hyperglycemic rats, only whole MC juice significantly decreased BG to the level that was quite similar to tolbutamide (~14–17%). Low dose of AF significantly reduced BG in both 2 and 4 hrs after its administration (15 and 24% respectively) whereas medium and high dose has less effect. However, AF had no hypoglycemic action in STZ-induced hyperglycemic rats. Both whole MC juice and low dose AF reduced area under curve of glucose in OGTT study. In conclusion, the highest hypoglycemic activity of Momordica charantia is associated with whole fruit in both normoglycemic and hyperglycemic rats. Arcangelisia flava Merr. also reduces blood glucose but the magnitude is lower with increasing dose. No hypoglycemic action of Arcangelisia flava Merr. is shown in STZ-hyperglycemic rats whereas low dose improves oral glucose tolerance.

P 17: Hypoglycemic Action of Red and White Osmium sanctum Linn. in Normoglycemic and Hyperglycemic rats.
Suksa-ard J², Songsak T², Palakul T² and Suanarunsawat T¹
Osinum sanctum Linn. (OS) has been shown to reduce blood glucose (BG) in both normal and diabetes mellitus. However, there are two types of OS in Thailand, and it is not known which one exerts hypoglycemic action. The present study was performed to elucidate the glycemic effect of both red and white OS in normoglycemic and hyperglycemic rats. Hyperglycemia was induced by either oral glucose loading (5 g/kgBW) or streptozotocin (STZ) injection. Both fresh and dry leaves of red and white OS were extracted by 95% ethanol. After overnight fasted, rats were fed by both preparations of red and white OS (400 mg/kgBW) and tolbutamide (90 mg/kgBW). Blood was collected from tail vein to determine BG before and after 2-4 hrs of drugs administrations. Oral glucose tolerance (OGTT) was also undertaken. The results show that both fresh and dry leaves extracts of white OS reduced BG throughout 4 hrs periods (17-23%, P< 0.01) whereas less but significant hypoglycemic effect was shown in STZ-induced hyperglycemic rats. Hypoglycemic level of fresh and dry leaves extract of white OS was quite the same, but slightly less than tolbutamide. Neither fresh nor dry leaves extract of red OS produced hypoglycemic action. During OGTT, area under curve of glucose was significantly improved by both fresh and dry leaves extracts of white OS. It can be concluded that only white Momordica charantia has hypoglycemic action in both normoglycemia and hyperglycemia. No significant difference of hypoglycemic activity between fresh and dry leaves extract of white Momordica charantia.

P 18: Vasorelaxant Effect of Morus alba Extract on L-NAME Hypertensive Rat Aorta
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Objective: The present study aims to examine the relaxant effect of M. alba extract on isolated thoracic aorta of N-nitro-L-arginine methyl ester (L-NAME) hypertensive rat. Background: Morus alba (M. alba) is used in folk medicine for a variety of disorders for instance; heart disease, asthma, hyperglycemia, inflammation, infections, tumors and oxidant stress. It has also been claimed to possess antihypertensive property, however, its effect on the vascular tone of the artery has not been examined.
Materials and Methods: Hypertension was induced in the male Sprague-Dawley rats by giving L-NAME 50/mg/kg/day in drinking water for 3 weeks. On experiment day, hypertensive state was verified by measurement of arterial blood pressure through femoral artery. The thoracic aortas were isolated and cut into ring segments of 3 mm length and mounted in 15 ml organ bath containing a physiological salt solution at 37 °C and gassed with mixture of 95% O2-5% CO2. The vascular relaxation effects of M. alba both water and ethanolic extracts (10-1000 µg/ml) were tested. Epigallocatechin gallate (EGCG; 0.05-137 µg/ml) was used as positive controls. In separated set of experiments, glybenclamide, a specific K+ channel blocker was used to determine the role of ATP-mediated K+ channels in the vasorelaxant response of M. alba.
Results: The extract of M. alba induced vasorelaxation with IC$_{50}$ of 242 ± 53 and 324±66 µg/ml and maximal relaxation of 99±0.8% and 90±4.1% for the water extract and ethanolic extract, respectively. For that of EGCG was 4.7±1 µg/ml and 67±5.3 %. It appeared that glybenclamide did not influence the relaxation of M. alba extracts.

Conclusion: The present results suggest that M. alba both water and ethanolic extracts can relax the smooth muscle of L-NMAE rat aortas and its vasorelaxation may not mediated through the ATP-sensitive K$^+$ channel.

P 19: Localization of PTHrP in Pregnant and Lactating Rat Mammary Tissues
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Parathyroid hormone-related protein (PTHrP) was identified in the 1987 as a tumor product that cause of humoral hypercalcemia of malignancy (HHM) (1). In addition, its production by tumors, PTHrP is widely expressed in a variety of embryonic and adult tissues. PTHrP has an amino-terminal sequence similar to PTH and it can interact with PTH/PTHrP receptor (PTH1R) (2). The active mammary gland of rat and bovine secrete a significant amount of this peptide into milk during lactation period. The functions of the peptide in active mammary gland could relate to cell cycle turning on, cell proliferation and/or calcium homeostasis within the gland. Up to date, cell types in lactating mammary tissue that synthesize, store, and secrete PTHrP have not been evaluated. We, therefore, used immunohistochemistry technique to localize antigen, which immunoreacts with anti-hPTHrP polyclonal antibody, in various stages of rat mammary tissues, -7, 0, +7, +14 days as 0 representing a pupping day. The immunostaining of PTHrP was detected in cytoplasm of mammary epithelial cells, stromal fibroblast, myoepithelial cells and smooth muscle cells. The intensity of positive PTHrP immunostaining was analyzed by UTHSCSA image tool. We found that during mammary gland development, the PTHrP immunoreactivity shown the lowest intensity. The stronger intensity was detected in mammary tissue which starts to active (0 day) and being lactating (+7 days). However, the PTHrP immunoreactivity intensity decreased dramatically in mammary tissue at peak of lactation (+14). These could be because of increasing PTHrP secretion into milk at this period (3).