College of Biochemists of Sri Lanka

Proceedings of the First Bi-annual Conference

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"Biochemistry for Sustainability"

Conference Editors

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[Registration number: GL00205278]

[Affiliated to Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB)]

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First Bi-annual Conference2018

Programme

Time	26th October 2018
8.00 - 8.30 a.m.	Registration
8.30 -9.45 a.m.	**Inauguration** **Keynote address by Prof. Lim Yang Mooi** *Centre for Cancer Research, Department of Preclinical Sciences, Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, Sungai Long Campus, Malaysia*
9.45 -10.15 a.m.	**Refreshments and Networking**
10.15-10.45 a.m.	**Video presentation of Prof. I. Parker,** University of Cape Town, South Africa
10.45 -12.15 p.m.	**Symposium 1** **Biochemistry, Nutrition and Health** Prof. Pujitha Wickramasinghe, University of Colombo Prof. Kamani Jayathilake, University of Ruhuna Prof. Lal Chandrasena, University of Kelaniya
12.15-12.40 p.m.	**Plenary Lecture1: by Prof. Anthony Ho Siong Hock** *Taylor's University, Subang Jaya, Malaysia*
12.40 - 1.20 p.m.	**Lunch** Lunch-time talk by Delmege Forsythe & Co. Ltd Healthcare Cluster
1.20 - 1.55 p.m.	**Poster Viewing**
1.55 - 2.45 p.m.	**Oral Presentations**
2.45 - 3.15 p.m.	**Plenary Lecture 2: by Dr. Ahmed Fahmi,** UNESCO
3.15 - 4.45 p.m.	**Symposium 2** **Biochemistry for Future** Prof. Preethi Soysa, University of Colombo Prof. Hemantha Peiris, University of Sri Jayewardenepura Prof. Shirani Ranasinghe, University of Peradeniya
4.45 - 5.00 p.m.	Awards & Closing Ceremony
5.00 p.m.	**Refreshments & Networking**

Message from the President of CBSL

I consider it an honour to be delivering the President's message for the First Biannual Conference of the College of Biochemists of Sri Lanka (CBSL), which was established in 2013 with the aim of setting up a platform for Sri Lankan Biochemists and Molecular Biologists for uplifting academic, educational and research capabilities. We were able to organize a very successful symposium on "Biochemistry: Towards Academic Excellence" in June 2016 in collaboration with Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB) of which CBSL is a constituent member.

The theme of today's conference; '**Biochemistry for Sustainability'** was selected to emphasize on the role of Biochemists as well as Molecular Biologists who would be the driving-force for future sustainability. The keynote address, symposia and plenary lectures highlight the potential research needs of Sri Lanka and the best possible measures to address these issues in future. While welcoming all the participants for the conference, I extend my deep gratitude to all the speakers; local and foreign, for accepting our invitation to deliver lectures at the conference.

It is my sincere wish that the young participants would take the advantage of the opportunity to meet with world-renowned local and foreign Biochemists and Molecular Biologists and develop collaborations and networks for the advancement of Biochemistry in Sri Lanka.

I appreciate the generous contribution by our sponsors, without whom, a conference of this magnitude is not possible. Finally, I wish to extend my profound gratitude to the members of the Organizing Committee who have made a tremendous effort to make the conference a success.

Prof. Sugandhika Suresh,
President,
College of Biochemists of Sri Lanka,
Sri Lanka.

Chemo-preventive Phytochemicals in Regulating Cancer Signaling Pathways

Prof Lim Yang Mooi

Centre for Cancer Research, Department of Preclinical Sciences, Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, Sungai Long Campus, Jalan Sungai Long, Cheras, 43000 Kajang, Selangor, Malaysia

Cancer is still named as the top killer disease worldwide with an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018. The contributing factor to the challenge in cancer treatment and the high mortality rate is the complexity of carcinogenesis, which is regulated by multiple signaling pathways. Thus, regulating multiple molecular alterations at the molecular level by chemopreventive phytochemicals will be a promising approach in delaying cancer formation. Thus far, dietary phytochemicals such as curcumin, resveratrol, isothiocyanates, (-)-epigallocatechin gallate, lycopene, sulforaphane, quercetin and retinol are regarded as the promising chemo preventive agents. These phytochemicals has been demonstrated to positively regulate various dysfunctional cancer signaling pathways that execute the cellular processes such as cell proliferation, cell survival, cell cycle regulation, inflammation, angiogenesis and metastasis. In this talk, the chemopreventive properties of maslinic acid targeting at protein kinase C and its downstream transcriptional factors of NF-κB and AP-1 via inflammatory pathway will be revealed.

Molecular Mechanisms of Cytotoxicity of Garlic Derived Ajoene and its Analogues on Cancer Cells

Prof M. Iqbal Parker

Departments of Medical Biochemistry and Structural Biology, University of Cape Town, Observatory, South Africa

Garlic is a food and medicinal plant that has been used in folk medicine since ancient times for its beneficial health effects, which include protection against cancer. Crushed garlic cloves contain an array of small sulphur-rich compounds such as ajoene. Ajoene can interfere with biological processes and is cytotoxic to cancer cells in the micromolar range. BisPMB is a synthetic ajoene analogue that we have synthesized and have shown in our lab to have superior cytotoxicity to ajoene. DNA micro array analysis of bisPMB-treated WHCO1 oesophageal cancer cells identified several pathways that are affected by bisPMB. The most significantly enriched biological processes as assessed by Gene ontology, KEGG and Ingenuity Pathway analysis were those involving protein processing in the endoplasmic reticulum (ER) and the unfolded protein response. In support of these pathways, bisPMB was found to inhibit global protein synthesis and lead to increased levels of ubiquitinated proteins. BisPMB also induced alternate splicing of the transcription factor XBP-1; increased the expression of the ER stress sensor GRP78 and induced expression of the ER stress marker CHOP. CHOP over-expression was found to be central to the cytotoxicity of bisPMB as its silencing with siRNA rendered the cells resistant to bisPMB. The MAPK proteins, JNK and ERK1/2 were activated following bisPMB treatment. Although JNK activation was not found to be critical in the cytotoxicity of bisPMB, ERK1/2 activation played a pro-survival role. Overall the ajoene analogue bisPMB appears to induce cytotoxicity in WHCO1 cells by activating the unfolded protein response through CHOP.

The Changing Face of the Biotechnology Workforce in the 21st Century

Anthony Ho S.H., PhD
Pro Vice-Chancellor, Research & Enterprise, Taylor's University, Subang Jaya, Malaysia

What we know about biotechnology and how we have used the tools of biotechnology has transformed over the last three decades. Sectors such as healthcare, pharmaceuticals, food, agriculture, advanced manufacturing, environmental sustainability are a few examples where biotechnology has made a lasting impact and arguably, a positive effect on the fate of humankind. While the traditional model of education has served us well in producing able workers for the industries where biotechnology is used today, there is increasing evidence that the pace of change in the industry is outpacing the capability of the workforce to adapt and change accordingly. Furthermore, the skill-sets of the individual deemed important yesterday are no longer sufficient today and will be woefully inadequate for the future. As civilization advances, solving problems will demand a scientific solution taking into account humanistic values, culture and even religion. The biotechnology industry and by extension the people involved in it, must embrace a much wider repertoire of skills and move beyond science and engage the arts, social sciences and humanities. This paper challenges how we educate ourselves today to recognize, analyze and express problems and how we need to bring together teams of people, with differing skills in order to create solutions that are meaningful. Questions such as 'What are we teaching?', 'How are we learning?', What values are we imparting?', 'How do we measure the success of these initiatives?', 'How do the art and humanities augment, motivate and influence the sciences?', 'Is the traditional university model outdated?' will be explored. For biotechnology to remain relevant it must incorporate multidisciplinary thinking in order to comprehensively solve the needs of society in the coming years.

UNESCO Programmes in Biotechnology

Dr Ahmed Fahmi, UNESCO

After the adoption of Agenda 2030 by the United Nations, a number of technologies have been proposed to address some of the Sustainable Development Goals (SDGs). Biotechnology is consistent with the SDGs, but it depends on how the technology is being used. Is it being used under regulatory bioethical standards that govern the safety of this technology for public use as well as its effect on the environment and on public acceptability. If one looks at the SDGs Goal 3 on ensuring health for all and Goal 2 on ensuring food security, one sees that countries have agreed that scientific technologies used in developing vaccines and better crops, respectively, should be utilised to implement these objectives. The issue of use of any technology is subject to each countries law and policies. In all high-end technologies such as biotechnology, scientific research continues at a rapid pace, and this has given rise to two major concerns. One concern is that the poorest and least technologically-developed countries will fall so far behind in developing capacity in biotechnology that its potential benefits will bypass those populations who have the greatest need. The other concern is that, because of this same lack of capacity to manage technical change, these populations will be the most vulnerable to potential misuse of this technology and have little control on the foreign private companies that operate this technology in their countries. The issues of technology access, facilitation and Intellectual Property Rights, are still on-going processes that are debated between the North and South countries. UNESCO had been playing an important role since the late 1970s in giving a comprehensive world view of biotechnology, partly, through high level policy debates and partly through capacity building activities such as the Microbial Resource Centres Network, which provided a global infrastructure incorporating national, regional, and over 70 international co-operating laboratories geared to the management, distribution, and utilization of microbiology; the UNESCO Biotechnology Education and Training Centres (as Category II centres), UNESCO chairs in biotechnology, the World Library of Science; a free online interactive platform providing access to high quality educational resources in biotechnologies and related fields for all communities across the globe, as well as many prestigious awards such as for example the UNESCO-L'Oreal award for women in science, a programme which was established to narrow the gender gap in the life and physical sciences. In fact UNESCO is the only unit within the UN that covers within its mandate the issue of "basic science for biotechnology", capacity building and science education. It therefore provides the vital basis for and complements work done by the FAO (agriculture) and WHO (medical applications). UNESCO is also one of the main UN organisations that debates the multidisciplinary and multicultural dimensions of biotechnology through its International Bioethics Committee, the Intergovernmental Bioethics Committee and also acts as the Secretariat of the United Nations Inter-Agency Committee on Bioethics.

Lipids in Human Health

Prof. Pujitha Wickramasinghe
Senior Professor in Paediatrics, Faculty of Medicine, University of Colombo

Lipids are important components of food, which perform an essential role in human health. Types of lipids are more important than the amount. Functional lipids are important for the prevention and treatment of many diseases. Omega-3 and omega-6 fatty acids, conjugated linoleic acids, medium chain triglycerides, and phytosterols have many beneficial effects on human health.

Dietary consumption of the essential fatty acids, linoleic acid (LA; ω-6) and α-linolenic acid (ALA; ω-3) is necessary for human growth and development. Evidence suggests a strong association between nutrition during the first 1000 days of life and cognitive development. In childhood, ω-3 PUFAs have been shown to contribute to ongoing cognitive development and may be involved in metabolic programming of bone turnover and adipogenesis. ω-3 PUFAs may also play an important role in adult neurophysiology and disease prevention.

The earliest reports and epidemiological studies revealed that the traditional Greenlandic diet rich in marine mammals and fish, reduced the incidence of cardiovascular disease in the Inuit population and Danish settlers significantly, but to different levels. Several studies have shown that ω-3 PUFAs play a significant role in altering blood lipid profiles and membrane lipid composition and affect many other functions such as eicosanoid biosynthesis, cell signaling cascades, and gene expression which in turn influences human health. In addition, the beneficial effect of ω-3 PUFAs are seen in patients with many diseases, such as cardiovascular disease, diabetes, cancer, depression and other psychiatric illnesses, periodontal disease and rheumatoid arthritis.

Fatty acids, originate primarily from plant sources either land or marine. Algal, and single-cell sources are a major contributor to the primary source of fatty acids in the food chain in addition to a few other land sources. Subsequently long-chain (LC) ω-3 PUFA are found in fatty fish, the liver of white lean fish and the blubber of marine mammals. Fish oils are sold as ω-3 PUFA supplements or in a concentrated form as ethyl esters (EEs) or acylglycerols, whereas algal, fungal, and single-cell oils have recently become popular as novel and renewable sources of LC ω-3 FA. In addition, krill oil contains both triacylglycerol (TAG) and phospholipid (PL). Although marine organisms are the major source of ω-3 PUFAs, some plant seeds also contain them.

Maternal docosahexaenoic acid (DHA) supplementation has suggested to be linked with cognitive development of their offspring. DHA is a structural component of human brain and retina.

Reactive Oxygen Species Signaling Cascades and Antioxidants

Prof. Kamani Jayatilaka
Professor of Biochemistry, Faculty of Medicine, University of Ruhuna

Reactive oxygen species (ROS) are produced as a result of cellular metabolism and in response to exogenous sources. Oxidative stress refers to the imbalance due to excess ROS generation exceeding the capability of the cell to produce an effective antioxidant response. Over the past three decades, there has been significant interest in oxidative stress and its role in the cellular dysfunction and in the pathophysiology of metabolic diseases. It has become apparent that ROS also serve as signaling molecules to regulate biological and physiological processes. The aim is to explore the pattern of the generation ROS, the mechanisms and targets of ROS on cell-signaling and to discuss how the cellular redox environment is preserved by enzymes and antioxidants.

Antioxidants in Cataracts and Reperfusion of Cardiomyocyte

Prof. Emr. L G. Chandrasena
Professor Emeritus, Biochemistry, University of Kelaniya

A free radical is an atom that contains an unpaired electron and this configuration renders free radicals highly unstable and chemically reactive. There is strong evidence for the role of free radicals in a wide variety of diseases and degenerative conditions including pathogenesis of cardiac disease, cancer and cataract. Oxidative stress may result when the cellular antioxidant defense mechanisms are unable to keep pace with the detoxification of reactive oxygen intermediates (ROI). ROI mediated peroxidation of membrane lipids can cause extensive damage to proteins leading to irreversible deleterious effects. In this context, the erythrocyte antioxidant model was used to study the pathogenesis of cataract and ischemic injury during coronary artery bypass surgery (CABG).

Oxidative stress and Cataract

Cataract is one of the leading causes of blindness in the world today. The health of the lens depends greatly on the reducing state of proteins in the lens. Cataracts develop due to a) concentrated action of accumulation sorbitol within the lens and b) the oxidized state of the lens proteins (crystallins) in diabetics (osmotic cataract). Our previous studies have revealed that erythrocyte antioxidant enzymes, G6PD, GSH, catalase, GPX, SOD and lipid peroxidation rate are sensitive indicators for the changes taking place in pathogenesis of cataract. G6PD deficient subjects are prone to develop cataract and if G6PD deficient patients are diabetic they are more vulnerable to cataract formation. Hence, the RBC antioxidant enzymes could be used as markers to identify persons at risk of developing cataract.

Oxidative stress and Reperfusion injury during CABG

Several mechanisms and mediators of reperfusion injury have been described. The most frequently cited are oxygen free radicals and microvascular dysfunction and altered myocardial metabolism. Molecular oxygen, when reintroduced into the ischemic myocardium following reperfusion leads to formation of oxygen free radicals. These reactive oxygen species (ROS) frequently cause initial cell injury. Overproduction of ROS are neutralized by various antioxidant mechanisms and amongst them, glutathione peroxidase (GPX) and superoxide dismutase (SOD) have been reported to be more sensitive cardio protective enzymes during ischemia- reperfusion injury.

There is substantial evidence to show that reperfusion results in the release of antioxidants due to overproduction of ROS such as superoxide anions and hydroxyl radicals. These antioxidants are a defense response to increased levels of cellular oxidation to minimize oxidative damage during reperfusion. Therefore, monitoring of oxidative stress during reperfusion may be of value in minimizing postoperative complications.

Cell Based Assays in Natural Product Drug Discovery

Prof. Preethi Soysa
Professor in Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo

Natural products from plants and animals cater throughout the ages for medicines for treatment of a wide spectrum of diseases. The most dominant source for natural medicine is plants. The prescriptions have evolved as a result of experimenting by trial and error for hundreds of centuries. Animal studies are the most common method for screening of natural products in drug discovery. However, the validity of the results is obscure, attributed to biological differences between humans and animals. Animal cell culture is a better option to replace unnecessary use of animals in screening of broad spectrum of natural products in drug research. As alternatives, cell-based studies with precision cut porcine liver slices from slaughter house, biopsy samples, immortalized cell lines, primary cell lines and embryonic stem cells or induced pluripotent stem cells are being used. The ability to re-programme / differentiate to other cells, make stem cells a valuable tool in the current drug discovery processes. Monolayer (2D) cell culture provides a wealth of information in drug discovery. The architecture and microenvironments of cells *in vivo* are different in many aspects from 2D cell culture. In contrast, the 3D cell culture is physiologically more compatible with the cell microenvironments *in vivo*. These include multicellular spheroids, organoids, scaffolds or scaffold free systems and hydrogels. The development of appropriate *in vitro* models could minimize animal experiments in screening of natural products and their secondary metabolites in drug discovery.

Novel Biomarkers of Kidney Disease: Current Status and Future Challenges for Sri Lanka

Prof. Hemantha Peiris

Senior Professor of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura

Chronic kidney disease of unknown aetiology (CKDu) is recognized as a major non-communicable disease of growing epidemic worldwide. Overall CKD mortality has increased by 31.7% over the last 10 years, making it one of the fastest rising major causes of death.

In the last decade, CKDu has emerged as a significant contributor to the burden of chronic kidney disease (CKD) in the North Central Province (NCP) of Sri Lanka, which is located south of India rural Sri Lanka. The disease is not due to conventional risk factors such as diabetes, hypertension and chronic glomerulonephritis. However, it is likely due to interactions of various potential agents such as heavy metals, pesticides, microbial toxins, chemicals, hard water mediated CKD and presence of tubule-intestinal pathology on renal biopsy which further supports the theory that the aetiology of this illness is multi-factorial.

There are limitations in using conventional screening laboratory methods such as serum creatinine, eGFR and urinary albumin–creatinine ratio have limitations with respect to sensitivity, specificity, and timeliness of diagnosis. Moreover, when the diagnosis is made, many patients are already requiring renal dialysis. Thus, it is the time now to look for more sensitive and specific panel of novel biomarkers which can be used for early detection of acute injury in CKDu patients.

Urinary Biomarkers provide major advantages in monitoring AKI or CKD compare with serum markers due to non-invasive, larger volumes compensate for the lower concentration of proteins and peptides in urine compared with plasma. Excretion patterns of some biomarkers may be useful in identifying nephrotoxicant induced injury in the CKDu patients. Most of the urinary markers are soluble proteins or other biomolecules, such as Kidney Injury Molecule-1 (KIM-1), β_2 – macroglobulin, Cystatin C, IL-18 and Neutrophil Gelatinase-Associated Lipocalin (NGAL) have been reported to be more sensitive and early markers of acute kidney injury. Hence, the new panel of urinary biomarkers will likely become more potential tests for screening and identifying patients with CKDu in the future. To be prepared for the future, obviously the most important challenge is to validate its use as a profile test rather than a single marker test.

The Role of Minerals for a Healthy Life

Prof. Shirani Ranasinghe
Professor of Biochemistry, Faculty of Medicine, University of Peradeniya

The subject Biochemistry focuses on understanding how biological molecules, the elements there give rise to the processes that occur within living cells and between cells. Electrolytes and metals in human body are important areas with respect to metabolism and the disorders of metabolism. In my lecture, some important clinical disorders related to mineral and electrolyte experienced in my research would be discussed.

Gallstone (GS) is one of the leading upper gastrointestinal surgical problems causing a significant health care burden. Cholesterol, calcium bilirubinate, calcium phosphate and calcium carbonate are considered as common constituents in GS. Black pigment GS was significantly common among patients with type II diabetes mellitus. Further, all the patients with chronic haemolytic anaemia and alcoholic cirrhosis had black pigment GS. Gender, ethnicity and body mass index can be used to predict the formation of mixed cholesterol GS and black pigment GS.

Beta thalassaemia is a common monogenic hereditary haemoglobinopathy which is associated with compound complications. Zinc deficiency is commonly observed in thalassaemia patients, which is also associated with multiple health complications.

Urolithiasis is defined as any calculi originated within urinary system which consists of kidney, ureter, bladder and urethra. Calcium is the most abundant cation in all regions and several elements were detected in the nucleus area compared to the peripheral area of each stone. In chronic kidney disease of unknown aetiology (CKDu) fluorideis an aetiological factor. High fluoride levels in drinking water in relation to the prevalence of CKDu in Sri Lanka were investigated using rats as an experimental model. Long term exposure of manganese and its impact on dopamine level was investigated as an aetiological factor Parkinson's disease. The lowering of dopamine and increasing the dopamine sulphate were confirmed.

The presence of heavy metals, mercury and arsenic in ayurvedic medicine was investigated with respect to the health hazard and toxic effects on major organs were not observed at proper dosage levels.

The minerals and electrolytes play an important role in body functions and the body has the capacity to maintain the homeostasis in healthy individuals. But probably with unfavourable environmental factors including diet, polluted air, and water, the capacity of homeostasis can exceed which gives rise to many health problems. Minerals play an important role for a healthy life!

OP-1

Multiple Micronutrient Deficiencies Among Women Attending MOH Clinics in Colombo Municipal Area at Early Pregnancy

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Background: Optimum periconceptional micronutrient status ensures optimum foetal developmental and placental function. Data on multiple micronutrient deficiencies at early pregnancy is limited.

Objective: We aimed to assess the micronutrient deficits in women attending antenatal clinics at early pregnancy and associated risk factors.

Methods: Apparently healthy, 110 pregnant women aged 18-36 years at <12 weeks of gestation who have not started nutritional supplements were randomly recruited from randomly selected maternity clinics in Colombo Municipal Council area. The study was approved by the Ethics Review Committee, Faculty of Medicine, Colombo. Haemoglobin [Hb], serum ferritin [SF], retinol and red cell folate were measured by haematology analyser, enzyme linked immunosorbent assay [ELISA], high performance liquid chromatography [HPLC] and chemiluminescent assay respectively. Inflammatory status was assessed by high sensitive C-reactive protein using immunoturbidometric assay and used to calculate a correction factor to adjust SF values for inflammation.

Results and Discussion: Among the 110 women studied, 11.8% had anaemia (Hb<11.0 g/dL), 70.9% had low iron stores (SF<30 µg/dL), 6% (n=7) had low vitamin A stores (serum retinol<46µg/l) and 48.2% had low folate stores (red cell folate<151 ng/mL). Eighty five percent (94/110) of women were deficient in at least one micronutrient. Among them 42% (46/94) had multiple micronutrient deficiencies. Nearly 32% (35/110) of the women studied were pregnant for the first time. The risk of having multiple micronutrient deficiencies at early pregnancy significantly increased (OR): 3.993 [95%CI; 1.465, 10.881]) when women had been pregnant for more than one time, ~~a~~after adjusting for the confounding factors (age, body mass index and dietary intake of iron, folate and vitamin A).

Conclusion: Among these women in their early pregnancy, over two fifth had multiple micronutrient deficiencies, while a significant proportion had low iron and folate stores. Multiparous women are at higher risk of developing multiple micronutrient deficiencies at early pregnancy than the nulliparous women who were pregnant for the first time.

OP-2

Inhibitory Effect of the Leaf Extract of *Acronychia pedunculata*on Prostaglandin E_2 Level of Wistar Rats with Chronic Arthritis

WMKM Ratnayake[1], TS Suresh[1*], AM Abeysekera[2], N Salim[3], UG Chandrika[1]
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Background: Prostaglandin E_2 (PGE_2) is a mediator associated with physiological and pathological conditions. Although it has various physiological roles at normal level, alterations in PGE_2 are associated with pathological conditions such as inflammatory diseases. *Acronychia pedunculata* ("*Ankenda*" in Sinhala) leaves have been used for centuries in traditional medicine for the treatment of inflammatory diseases.

Objective: All our previous findings contributed to solicit the anti-inflammatory activity of this plant. As PGE_2 is an inflammatory marker, an attempt was made to evaluate inhibition of PGE_2 level by leaf extract of *A. pedunculata* on adjuvant-induced arthritis rat model, in the present study.

Methods: Healthy adult male, Wistar rats (150-200 g) were used for the experiment (n=6/group). The negative and positive control groups were orally administered with 1.0 mL of 0.5 % carboxymethyl cellulose and celecoxib (20 mg/kg b. w.) respectively. The test group received a dose of 200 mg/kg b. w. of 70 % ethanol extract of *A. pedunculata* leaves (EEAL) which was found to be the most effective dose during the studies on acute anti-inflammatory activity. The oral treatments were started on day 14 of the experiment, continued to day 28.

Results and Discussion: Treatment with 200 mg/kg b. w. of EEAL significantly ($p < 0.05$) inhibited the PGE_2 level as compared to the negative control. It was 266 ± 44 pg/L, in EEAL treated group whereas it was 824 ± 83 pg/L for negative control. PGE_2 level for celecoxib treated group was 306 ± 36 pg/L.

Conclusion: The present study demonstrates that the EEAL has PGE_2 inhibitory activity which may be contributing to its anti-inflammatory effect. The findings justify the traditional use of this plant in the treatment of various types of inflammation.

OP-3

Novel Method to Extract Genomic DNA from Gram-negative Bacteria

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***Background*:** Currently there are several protocols to extract bacterial genomic DNA. However, in most of these methods, hazardous organic solvents, including phenol and chloroform are used for deproteinization, whereas in certain protocols, expensive enzymes including RNases and proteinases are used. Most of these methods are time-consuming and expensive.

***Objective*:** This study was designed to introduce a simple, rapid, user-friendly, inexpensive and effective genomic DNA isolation procedure for Gram-negative bacteria, without the usage of toxic chemicals and costly enzymes. The second objective was to compare this novel method with the modified salting-out method.

***Methods*:** The bacterial cell pellet was re-suspended in TEN (Tris, EDTA, NaCl) and TENST buffer (Tris, EDTA, NaCl, SDS, Triton-X-100) and incubated. After digestion was completed, saturated NaCl was added and centrifuged for protein precipitation. The supernatant containing DNA was transferred and exactly 2 volumes of absolute ethanol was added, and the tubes were inverted several times. The tubes were centrifuged to precipitate DNA. The precipitated DNA was dissolved in 50 µl of TE buffer (Tris-HCl, Na_2EDTA) after discarding the supernatant. A modified salting-out method was also used to extract DNA from the same organism and these two methods were compared. Concentration and yield were determined after gel electrophoresis was completed by comparing the sample DNA intensity to that of a DNA quantitation standard.

Results and Discussion: Both methods were successful in extracting bacterial genomic DNA, however, the DNA yield was higher in the novel method. Absence of RNA was confirmed as there was no smeared band towards the bottom of the gel. The novel method has several advantages over other protocols, since it does not require any hazardous chemicals or expensive enzymes and is less time-consuming.

***Conclusion*:** The presented method is simple, non-toxic, inexpensive, rapid and efficient for routine DNA isolation from Gram-negative bacteria.

OP-4

Comparison of High Sensitivity C -Reactive Protein Levels Among Acute and Chronic Lower Back Pain Patients Undergoing Lumbar Discectomy

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***Background*:** Lumbar disc herniation (LDH) gives rise to low grade inflammation around the herniated discs. Level of high sensitivity C-reactive protein (hs-CRP) may associate with the severity of lower back pain.

***Objective*:** To identify the association between hs-CRP levels and acute back pain (ABP) and chronic back pain (CBP) in patients undergoing lumbar discectomy.

***Methods*:** A serum aliquot of 200 µL from each patient (n=104) undergoing lumbar discectomy was analyzed for hs-CRP using immunoturbidometric assay.

***Results and Discussion*:** Majority (81.7 %) presented with CBP (males=44; females=41) while 18.4 % had ABP (males=10; females=9). In both CBP and ABP groups, age ranged from 18-79 years. Even though a significant difference (p=0.211) was not observed in mean hs-CRP, CBP patients had (4.6±8.4 mg/L) elevated hs-CRP compared to ABP (2.1±2.5 mg/L). There were 32.9 % CBP patients with elevated hs-CRP (>3 mg/L). Studies have reported that hs-CRP in CBP remains constant with no correlation to the pain. However, 5/19 ABP patients had elevated hs-CRP (>3 mg/L) levels.

***Conclusion*:** High hs-CRP level in patients with CBP might be suggestive of low grade inflammation around the herniated disc and the necessity for anti-inflammatory treatments in CBP.

Acknowledgment

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OP-5

Genotyping of the LPL gene S447X Polymorphism and its Association with Serum Lipid Levels in Young Urban Women

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Background: Lipoprotein lipase (LPL) hydrolyses triglycerides in chylomicrons and very low-density lipoproteins (VLDL). S447X polymorphism is a single nucleotide substitution of Cytosine (C) with Guanine (G) in the LPL gene in exon9 which creates a protein truncation.

Objective: The objectives were to genotype the LPL gene S447X polymorphism and to determine its association with serum lipid levels among young urban women.

Methods: Apparently healthy women aged 18-30 years (n=90) were recruited from Colombo municipal area. Fasting lipid profile was analyzed using standard kits. LPL gene S447X polymorphism was genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results and Discussion: The genotype frequencies; 62 "CC", 26 "CG" and 2 "GG" and allele frequencies; 0.83 "C" allele, 0.17 "G" allele which was according to Hardy Weinberg equilibrium ($\chi 2=0.14$, P=0.70). Binary logistic regression analysis revealed that the CC genotype is significantly associated with low HDL cholesterol (below the population mean; <45.29 mg/dL, OR=4.10, P=0.005, 95% CI=1.54-10.92) and, high non-HDL (above the population mean;<121.08 mg/dL, OR=2.96, P=0.044, 95% CI=1.03-8.51) and triglyceride (above the population mean;<119.15 mg/dL, OR=3.59, P=0.031, 95% CI=1.13-11.42) levels. This effect was more prominent among overweight women (HDL; OR=10.25, P= 0.008, 95% CI=1.83-57.40, non-HDL; OR=6.13, P=0.020, 95% CI=1.33-28.21and triglyceride; OR=9.33, P=0.006, 95% CI=1.87-46.57). Our findings indicate that individuals with CC genotype in the study group appear to have a lower LPL activity leading to increased triglycerides and non-HDL cholesterol in the circulation, especially among the overweight women.

Conclusion: Presence of the CC genotype appears to increase the risk of having low HDL cholesterol, high non-HDL cholesterol and high triglyceride levels especially among overweight women.

PP-1

Cloning, Expression, and Purification of Recombinant Anti-diabetic Drug Target, Peroxisome Proliferator Activated Receptor Gamma (PPARɣ)

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Background: Thiazolidinediones are effective anti-diabetic drugs that elicit the hypoglycaemic effect through activating the nuclear receptor PPARγ. PPARγ has always been a focus in the search of novel therapies with fewer side effects to treat type II diabetes mellitus.

Objective: The objective of the present study was to establish an optimized protocol for *in vitro* production of the recombinant PPARγ, which could be used in natural product-based anti-diabetic drug discovery research.

Methods: The open reading frame of the mouse PPARγ isoform 2 cDNA (full-length and truncated) was PCR amplified and cloned into pET28 plasmid with an N terminal His_6 tag. Each construct was transformed into Rosetta 2 (DE3) *E. coli and* successful transformants were induced with isopropyl β-D-thiogalactoside (IPTG). A combination of metal affinity and cation exchange chromatography was performed to purify the protein.

Results and Discussion: DNA sequencing confirmed the successful cloning of PPARγ into pET28 vector. Recombinant PPARγ full-length construct did not yield a detectable amount of protein in any of the tested expression conditions. The truncated PPARγ (PPARγ 102; containing amino acids 102 – 505) construct poorly expressed at temperatures above 20 °C even with IPTG levels as low as 0.1×10^{-3} M. However, PPARγ 102 reasonably expressed, in the soluble form, with 0.5×10^{-3} M IPTG at 16 °C. His tagged PPARγ 102 was purified first using Ni^{2+} affinity chromatography and the protein was eluted around, 250×10^{-3}M imidazole. Subsequently, purification with cation exchange chromatography eluted the protein around 500×10^{-3} M NaCl with a reasonable purity as detected on SDS-PAGE.

Conclusions: Our results evidence the possibility of producing large quantities of pure recombinant PPARγ 102 protein using a cost effective and convenient expression system.

Acknowledgements: Financial assistance by University of Kelaniya RP/03/SR/04/02/01/2016

PP-2

Evaluation of *in vivo* Anti-nephrotoxic Activity of the Standardized Aqueous Root Extract of *Vetiveria zizanioides* (L.) Nash of Sri Lankan Origin

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Background: *Vetiveria zizanioides* (L.) Nash (Family: Gramineae), commonly known as *Savandara* is a medicinal plant, widely used in the management of kidney diseases in Sri Lankan traditional medicine.

Objective: The aim of the present study was to standardize the root powder of *V. zizanioides* and to investigate the anti-nephrotoxic activity of the standardized aqueous root extract of *V. zizanioides in vivo.*

Methods: The chemical standardization and qualitative phytochemical analysis were conducted following standard protocols. The anti-nephrotoxic activity was investigated in adriamycin (20 mg/kg, ip) induced nephrotoxic male Wistar rats (n=6/group). The lyophilized powder of the aqueous refluxed (4h) root extract of *V. zizanioides* was administered at three selected doses; 25, 50 and 100 mg/kg to nephrotoxic rats. Fosinopril sodium (0.09 mg/kg) was used as the positive control. The anti-nephrotoxic activity was determined using serum concentrations of creatinine, albumin, total protein and concentration of urine total protein. Further, assessment of histopathological changes in the kidney tissues of H and E stained sections was carried out. Ethical clearance was granted from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna (14.12.2015:3.1).

Results and Discussion: The root powder of *V. zizanioides* consisted of 7.5±0.4 % moisture, 30.2±0.9% total ash, 0.8±0.1 % water soluble ash and 26.2±0.9% acid insoluble ash. The preliminary phytochemical analysis of *V. zizanioides* extract revealed the presence of flavonoids, coumarins, tannins, saponin, phenolic compounds and terpenoids. The *V. zizanioides* extract at the 25, 50 and 100 mg/kg doses and fosinopril reduced the increase in serum creatinine concentration by 11%, 33%, 51% and 42% respectively ($p<0.05$). The serum concentration of albumin (3%, 13%, 20% and 16%) and total protein (9%, 20%, 32% and 12%) were increased significantly compared to the nephrotoxic control group ($p<0.05$). The reduction in the loss of urine total protein was found to be dose dependent with the selected doses of *V. zizanioides* in nephrotoxic rats. Histopathological assessment of H and E stained kidney sections showed an attenuation of nephrotoxic changes in the kidney tissues.

Conclusions: The results revealed that the standardized aqueous root extract of *V. zizanioides* possesses significant dose dependent anti-nephrotoxic activity in adriamycin induced nephrotoxic rats. The identified secondary metabolites might be attributed to the anti-nephrotoxic activity *in vivo.*

Acknowledgements: Financial assistance by NSF research grant (RG/2016/HS -03) and UGC special allocation for strengthening research (RU/PG- R/16/14)

PP-3

Induction of Apoptosis is Caused via Reduced Glutathione Level by *Clerodendrum infortunatum L.* Roots in Rhabdomyosarcoma (RD) Cancer Cells

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Background*: Clerodendrum infortunatum L.* roots are traditionally used in cancer treatment. However, the detailed mechanism has not been investigated.

Objective: The present study was carried out to investigate the anticancer potential via induction of apoptosis by decreasing Reduced Glutathione (GSH) levels.

Methods: The root extract of *C. infortunatum L.* was prepared as described by traditional medicinal practitioners in Sri Lanka. RD cells were treated with the root extract for 24 hours. Morphological features of apoptosis were assessed by Giemsa staining. GSH level and Rhodamine 123 were determined to evaluate the oxidative stress and mitochondrial transmembrane potential (MMP) respectively.

Results and Discussion: Characteristic morphological changes of apoptosis were observed with Giemsa staining at 50, 150, 250 µg/mL. GSH levels observed with the negative control (85.1 ± 0.6 µg/mL) was reduced to 71.2 ± 0.7 and 35.9 ± 0.8 µg/mL at concentration of 50 and 600 µg/mL respectively. Decrease in cellular GSH below a threshold level initiates mitochondrial apoptotic signaling. MMP plays a central role in apoptosis cascade and reduced MMP observed in treated cells further confirmed the cell death occurred via apoptosis.

Conclusions: The results obtained for the present study suggest that *C. infortunatum L.* root exerts anticancer activity by decreasing GSH and thereby lowering the MMP in RD cells and lays the basis for further evaluation of a new therapeutic approach in anticancer treatments.

PP-4

Biodegradation of Crude Oil by Natural Microbial Communities Comprising *Aspergillus* and *Bacillus Species*

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***Background*:** Petroleum contamination is a global concern and various microbial genera have been tested in bioremediation of crude oil contaminations worldwide.
***Objective*:** Present study aims to evaluate natural microbial communities in degradation of crude oil in liquid culture at different temperatures.
***Methods*:** Three natural fungal-bacterial communities, C1, C2, C3 (comprising different isolates of *Aspergillus* sp. and *Bacillus* sp.) previously isolated from a municipal landfill, were grown on Bushnell and Haas medium with 1% sterile crude oil and incubated for seven days at 30 °C and 40 °C, respectively. Experiments were done in triplicate. Disintegration / removal of the oil layer was visually observed by comparison to a negative control devoid of the microbial inoculum.
***Results and Discussion*:** The removal of the oil layer, after 7 days at 30 °C, was highest in C3 (no remaining oil was visually observed), whereas at 40 °C it was highest in C1 (almost all the oil removed). At both temperatures, C2 showed the lowest efficiency. The efficiency of C1 was similar at both temperatures.C3 formed a well spread fungal-bacterial biofilm over the oil-water interface at 30 °C compared to C1 and C2, which may explain its efficiency.
***Conclusions*:** The highest efficiency in biodegradation of crude oil was observed by C3 community at 30 °C, while C1 was also efficient at 30 °C. Therefore it can be concluded that C1 and C3 communities are suitable bioremediation agents at 30 °C, which is cost effective at local climatic conditions.

PP-5

The Phytochemical Composition and *In-vitro* Anti-oxidant Activity of *Rasna Sapthakaya* Decoction

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Background: *Rasna sapthakaya* decoction (RSD) is a mixture of seven herbs and it has been proven as a potent anti-inflammatory Ayurveda preparation. In Sri Lanka and India, the decoction is used to treat chronic inflammatory joint diseases.
Objective: The study was conducted with the aim of investigating phytochemical composition, *in-vitro* anti-oxidant activity, total polyphenol content and development of TLC pattern of conventional RSD.
Methods: The RSD was prepared according to the Ayurveda conventional method and freeze-dried sample was used for the analysis. The phytochemical screening was carried out using standard methods. The *in-vitro* antioxidant activity and total polyphenolic contents were analysed by ABTS radical cation decolorization assay and Folin Ciocalteu method respectively. The thin layer chromatographic finger print and densitogram of standard *RSD* decoction were developed with the solvent system; Dichloromethane: Cyclohexane: Ethyl acetate (6: 2: 1.2).
Results and Discussion: Alkaloids, Flavonoids, Glycosides, Polyphenols, Quinones, Saponins, Tannins and Terpenoids were present in the decoction. The anti-oxidant activity of RSD was 1440±21.30 µg/L TEAC/g of extract. The percentage inhibition (PI) against the radical formation was 39.4±4.04 %. The total polyphenol content of the RSD was 54.0±0.815 mg GAE/g of extract. The TLC fingerprint profile of RSD is similar in terms of R_f values and colours to the TLC fingerprint profile of the standard mixture of raw materials of the decoction.
Conclusion: The results obtained in this study have established that the RSD is rich in vital phytochemicals and showed potent *in-vitro* anti-oxidant activity with high polyphenol content. The TLC profile and the densitogram obtained could be referenced as a standard for future studies on the decoction.
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PP-6

Gender Differences in the Association between Serum Ferritin and Metabolic Risk Factors in 8-9-Year-Old Children in Colombo Municipal Area

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Background: Serum ferritin reflects the iron stores of the body. As an acute phase protein, serum ferritin increases with inflammatory conditions. Elevated serum ferritin levels are found to be associated with metabolic syndrome.
Objective: The objective of the study was to investigate the gender differences in the association between serum ferritin and metabolic risk factors among 8-9-year-old children.
Methods: A cross sectional study was conducted. School children (8-9 years) were selected (N=324; Boys-N=161) within Colombo Municipal area. Weight, height, waist circumference (WC), % body fat (BF) and metabolic risk factors [fasting (8-10 hours) blood sugar (FBS), insulin, total cholesterol (TC), HDL, triglyceride (TG), LDL], ferritin and high sensitivity C-reactive protein (hs-CRP) were measured. HOMA IR (Homeostatic model of insulin resistance) was calculated as [fasting insulin (μU/L) x fasting glucose (nmol/L)/22.5]. The children with hs-CRP levels (acute phase protein) >10 mg/L were excluded from this analysis. Comparison was done between low and high ferritin tertiles along with metabolic risk factors.
Results and Discussion: Ferritin showed significant correlation with %BF ($r=0.508$, $p<0.001$), WC ($r=0.533$, $p<0.001$), Waist: height (WHtR) ($r=0.525$, $p<0.001$), FBS ($r=0.192$, $p=0.021$), Insulin ($r=0.485$, $p<0.001$), IR ($r=0.491$, $p<0.001$), TC ($r=0.225$, $p=0.007$), TG ($r=0.307$, $p<0.001$), LDL ($r=0.228$, $p=0.006$), HDL ($r=-0.166$, $p<0.048$) among boys but only WHtR ($r=0.197$, $p=0.013$) showed such relationship in girls. Significantly high levels of metabolic parameters and Low HDL levels were noted in high ferritin tertile compared to the low tertile in boys, [%BF-37.40 (31.1,41.4) vs 20.31 (13.9, 29.0), FBS (mg/dL)- 97.0 (88.5, 102.7) vs 90.0 (86.0, 98.0), Insulin (μU/L) -7.21(3.6,13.2) vs 2.16(1.85,3.96), IR-1.7 (0.83,2.97) vs 0.49 (0.41, 0.92), TC(mg/dL)- 187.50 (169.7, 198.0) vs 169.0 (157.7, 189.5), TG (mg/dL)- 88.5 (71.0, 121.5) vs 61.5 (46.7,77.5), LDL(mg/dL) -120.6 (105.95,132.4) vs 109.0 (90.5, 120.2), HDL (mg/dL)- 44.0 (39.0, 50.2) vs 48.5 (42.7, 57.0)] and they were not significant among girls.
Conclusion: Serum ferritin is associated with metabolic risk factors among boys but not girls in this study population.

PP-7

Assessment of 25(OH)D Threshold in a Population of Pregnant Mothers

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Background: It is apparent that vitamin D deficiency is becoming an issue all over the world at an epidemic level and it is seen among many regardless of age, ethnicity or socio demographic factors. However, there is no definite agreement for the optimal level of active metabolite of vitamin D [25(OH)D].

Objective: Our objective was to assess Institute of Medicine (IOM) recommendations on 25(OH)D threshold in a population of pregnant mothers.

Methods: Ethical clearance was obtained from the Ethics Review Committee, University of Sri Jayewardenepura. Women (n=105) aged 18 years or more with singleton pregnancies in their 3rd trimester were invited for the study at the antenatal clinic at Colombo South Teaching Hospital. Women on vitamin D supplements, having serious medical problems (non-obstetric) and disabilities that could be related to bone metabolism were excluded. A pretested interviewer administered questionnaire was administered to collect information on socio-demography, nutrition, health, physical examination including anthropometry. A serum sample was taken and stored at -20 °C prior to measurement of 25-(OH)D by VIDAS® 25 OH vitamin D Total using the Enzyme Linked Fluorescent Assay (ELFA). It is very well correlated to the Liquid Chromatography-Mass Spectrometry reference method with cross reactivity of 100% with 25 (OH)D and 91% with Vitamin D2. The DRG (EIA-3645) Intact-PTH ELISA was employed for quantitative determination of intact-PTH in serum. Calcium, phosphate and ALP were analyzed with colorimetric method. Data were analyzed using SPSS version 15.0.

Results and Discussion: 25(OH)D, ALP, PTH, calcium and IP were 18.6±7.2 ng/mL, 193.6±172.0 U/L, 23.7±22.0 pg/mL, 2.3±0.2 mmol/L and 1.3±0.2 mmol/L respectively. Only calcium and IP were within the normal range for the whole population. Vitamin D deficiency (<12 ng/mL) was 20.2%. However, abnormal PTH (>66.5 pg/mL) was found only in 5% among the deficient. Thus we calculated the sensitivity (20%) and specificity (81%) of the IOM cut off level for our sample. Positive predictive value was 5%.

Conclusions: Secondary hyperparathyroidism was not prevalent among the vitamin D deficient population. Since, the positive predict value for the vitamin D test is low; there is a need to redefine the cut off values for vitamin D levels in our population.

PP-8

Comparison of Selected Methods for Urine Protein Determination and Standardization of Sulphosalicylic acid Precipitation Method

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Background: Sulphosalicylic acid precipitation method is widely used to detect proteinuria in many clinical laboratories. Despite the recommended concentration and volume ratio of SSA to urine for sulphosalicylic acid method, laboratories are using different sulphosalicylic acid concentrations.

Objective: The main objective of the present study was to estimate and compare urine protein concentration in each detection method (different concentrations of SSA) with pyrogallol red molybdate dye binding method.

Methods*:* Urine protein concentration was calculated using different selected concentrations (3%, 6% ,20%, 30%) of sulphosalicylic acid with 3:1 ratio of SSA to urine, relevant volumes for higher concentrations of SSA (i.e. volume corrected SSA) and pyrogallol red molybdate dye binding method. Grading pattern for colour change of urine dipstick strip test and turbidity of different concentrations of sulfosalicylic acid were also compared.

Results and Discussion*:* Different concentrations of sulphosalicylic acid methods showed a significant underestimation relative to the pyrogallol red molybdate dye binding method and there were significant differences in protein yield between different concentrations of SSA except low concentration (6%) with reference 3% SSA method. However there was no significant difference in protein yield from volume corrected method even in high concentration of sulphosalicylic acid with the reference method (3% SSA). But better correlation was found between pyrogallol red molybdate dye binding method and 3% SSA as well as in volume corrected methods for higher concentrations of SSA. Urine dipstick strip test method shows significant relationship with 3% SSA method compared to other concentrations of SSA.

Conclusion: The concentrations of proteins are significantly different when using different SSA concentrations other than the recommended 3% SSA with 3:1 SSA to urine volume ratio. When using different concentrations of sulphosalicylic acid for quantitative analysis of urinary protein the accuracy is low and thus not suitable for the detection and quantification of proteinuria. Dipstick strip test results are only compatible with 3% SSA method.

PP-9

In vivo Anti- nociceptive Activity of the Aqueous Extract of Flowers and Stalks of *Aponogeton crispus*

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Background: Anti-inflammatory, anti-oxidant, anti-cancer, anti-diabetic and thrombolytic activities are some of the already proven pharmacological activities of plants of genus *Aponogeton*. *Aponogeton crispus*; called *Kekatiya* in Sinhala is a Sri Lankan native aquatic plant. Pain is one of the cardinal features of inflammation. This plant has been traditionally used for anti-inflammatory disorders even though it has not been proven scientifically. In addition, it is consumed as a green leafy vegetable.

Objective: This study aimed to assess the anti–nociceptive activity of the aqueous extracts of flowers and stalk of *Aponogeton crispus* in Wistar rats.

Methods: *Aponogeton crispus* flowers and stalks were collected from Malabe and the aqueous extract of flowers and stalks combined, was prepared according to the standard ayurvedic method. Acetic acid induced writhing model in Wistar rats (3 groups with 6 rats in each) was used to determine the anti- nocicpetive activity. Nociception was induced by the intra-peritoneal injection of acetic acid (0.6%, 10 mL/kg) into the peritoneal cavity of rats. Rats in the group I served as control and were administered with 1.0 mL of distilled water. Group II was administered the standard drug of 100 mg/kg body weight of acetyl salicylic acid, group III served as test group and received the aqueous extract of *A. crispus* (90 mg/kg). All were administered orally 30 minutes before the intra-peritoneal injection. Number of abdominal constrictions produced in rats was counted cumulatively for 20 minutes, following a latency period of 5 minutes.

Results and Discussion: The number of mean writhes shown by the rats in the negative control group was 45.33 ± 5.0. The group given *A. crispus* extract showed 25.33 ± 3.5 mean writhes and the percentage reduction of writhes was 46.5 % ($p<0.01$). The standard drug acetyl salicylic acid showed 23.00±3 percentage reduction of mean writhes were 51.4 % ($p<0.001$). These results were statistically significant compared to the negative control group.

Conclusion: The present study provides the evidence for anti- nociceptive activity of the aqueous extract of flowers and stalk of *A. crispus* in the Wistar rat model.

Acknowledgement: University of Sri Jayewardenepura research grant ASP/01/RE/MED/2016/72

PP-10

Chronic Toxicity Assay of Hot Water Extracts of Stems and Leaves of *Psychotria sarmentosa* on Healthy Male Wistar Rats

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Background: *Psychotria sarmentosa* (Family: *Rubiaceae,* Gonika in Sinhala) has been used widely in indigenous medicine in the treatments of several inflammatory diseases. It is used in porridge preparation and is also eaten as a tempered salad. Although the aqueous extract of this plant has been tested for various effects, no studies have been conducted with the hot water extract.

Objective: The objective of the study was to assess long term effects of hot water extracts of stems and leaves (HWESL) of *P. sarmentosa* in healthy male Wistar rats.

Methods: The most effective dose (2039 mg/kg) of HWESL was predetermined in a dose-response assay. The rats in Test and Control groups (6 in each) were orally administered with this test extract and distilled water respectively for 42 consecutive days. The body weights were measured each week during the study. Rats were observed for abnormal behaviour, convulsions or other toxic effects throughout the assay period. After the last dosing on Day 42, 1 mL of blood was collected the lateral tail vein of each rat for biochemical analysis (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Creatinine, Gamma Glutamyl Transferase (Gamma-GT) and haematological (Haemoglobin) to detect liver and renal functions. Data were analyzed statistically by analysis of variance (ANOVA). Results with $p<0.05$ were considered as significant.

Results and Discussion: There were no significant changes in body weights with respect to the control. The dose of 2039 mg/kg of HWESL caused no significant changes in the tested enzymes as well as creatinine and haemoglobin, when compared to the control group. Further, no abnormal behaviours were observed in the rats during the study period.

Conclusion: The present study confirms that chronic consumption of the hot water extract of the edible part (stem and leaves) of *Psychotria sarmentosa* does not exert any possible toxic effects in Wistar rats.

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PP-11

Glycaemic Responses to Four Types of Bread Made Using Different Composition and Processing Methods

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Background: Bread made with commercially available premixes is available for public. However, there is modest information with regard to their health benefits and the glycaemic responses.

Objective: The objective of this study was to determine the glycaemic responses to four types of bread made using different composition and processing methods.

Methods: *Kurakkan* (finger millet) bread (KB), Multigrain bread (MB), and white sliced bread [(diesel oven) (WB)] were prepared according to the standardized recipes. Ordinary white bread [(wooden oven) (OB)] was purchased from market. A 50 g available carbohydrate portion of bread was given with 05 g of *lunumiris* (chili chutney) and glucose was taken as the standard food for determination of glycaemic indices (GI). Normal healthy volunteers (06 males and 06 females; 20-30 years, BMI- 18.5-23.5 kgm^{-2}) not under any medical treatment participated in the study. Blood samples were taken at fasting, 15, 30, 60, 90 and 120 min incremental area under the curve (IAUC) were calculated. Ethical clearance was obtained from the Ethics Review Committee, Faculty of Medicine, University of Kelaniya, The results were analyzed using t-test and Minitab (version 15), taking the 95% confidence interval.

Results and Discussion: The GI values (mean +SEM) of MB, KB, OB and WB were 76±2.6, 88±3.0,90±2.4, and 91±2.6 and Glycaemic Loads (GL) were 14 ±1.7, 18±2.2, 22±2.0 & 23±2.5 respectively. The blood glucose peak values of MB, KB, OB, WB and glucose were 7.5 mmol/L, 7.3 mmol/L, 8.0 mmol/L (low peak in ordinary bread even with high GI), 7.1 mmol/L and 8.3 mmol/L respectively and all the breads types peaked at 30 minutes.

Conclusion: All bread varieties belong to high GI (GI≥ 70) and multigrain bread and *kurakkan* bread belong to medium GL (GL = 10-20) and ordinary white bread and white sliced bread belong to high GL (GL ≥ 20). Thus there is a need to improve on the ingredients on especially multigrain bread to prepare a bread with a medium GI.

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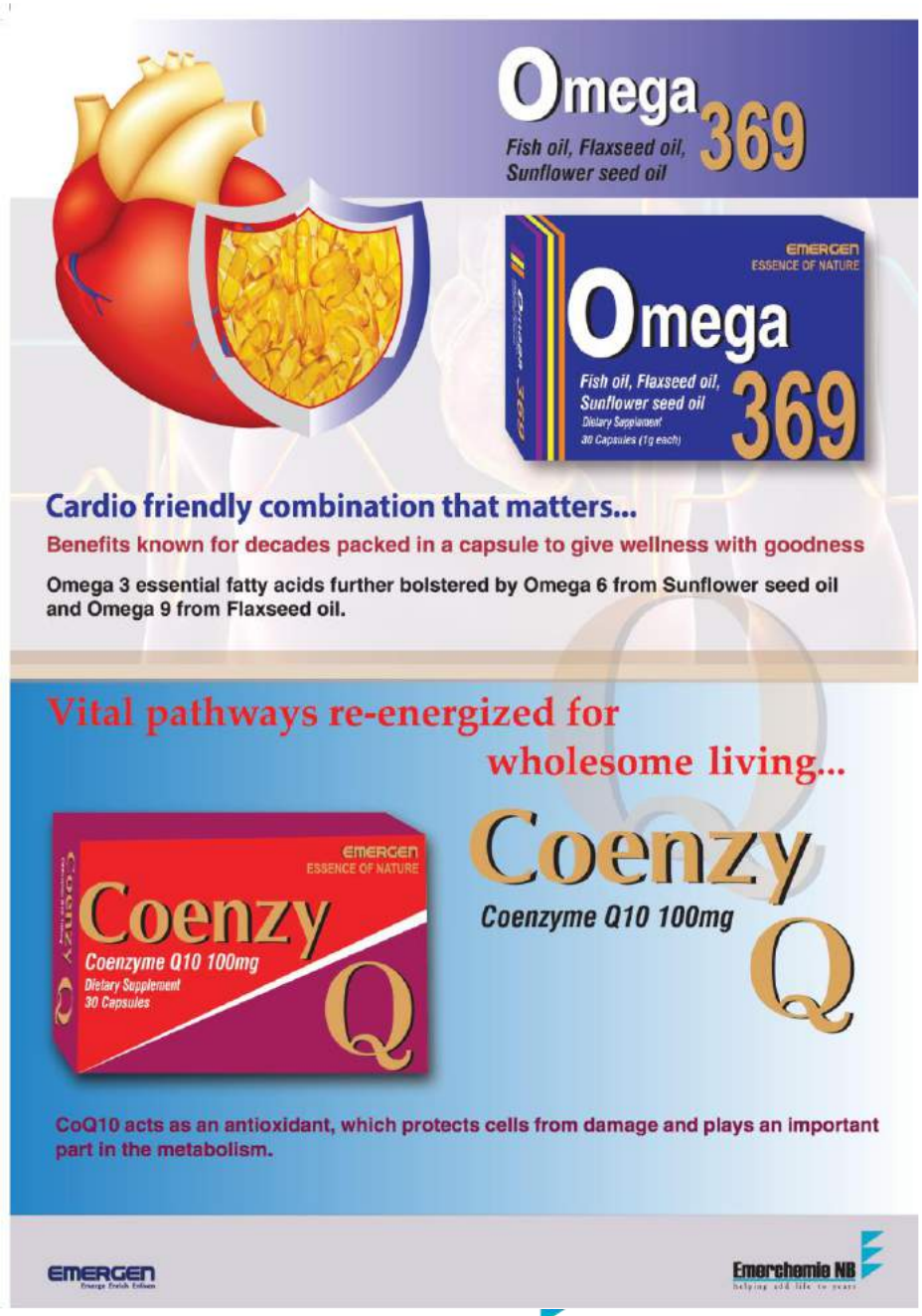

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