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Fourth Annual Scientific Sessions

5th May 2011

Programme and Abstracts

Institute of Biochemistry, Molecular Biology and Biotechnology

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Institute of Biochemistry, Molecular Biology and Biotechnology

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Our Vision

**" To be an
International Centre of Excellence
In
Molecular Life Sciences"**

PROGRAMME

09.30 h -	Inauguration
10.00 h -	**Professor Stanley Wijesundera Memorial Lecture** ***Drugs from Nature: Harnessing Chemical Diversity in Biodiversity*** by **Professor E. Dilip de Silva** Senior Professor of Organic Chemistry Department of Chemistry, University of Colombo
11.00 h -	Vote of thanks
11.10 h -	Reception
11.40 h – 12.40 h -	**Plenary Lecture: Next Generation Sequencing** By **Dr Erik Bongcam-Rudloff** Associate Professor, Swedish University of Agricultural Sciences, University of Uppsala, Sweden Former Chairman, European Molecular Biology Network
13.45 h – 14.45 h	Oral presentations: Session I (Cancer Genetics, Human DNA Variation and Reproduction)
14.45 h – 15.15 h	Oral presentations: Session II (Parasite and Vector Molecular Biology)
15.15 h – 16.15 h	Oral presentations: Session III (Plant Molecular Biology and Medicinal Plants)
16.15 h – 17.15 h	Tea and Viewing Poster presentations

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Message from the Director/ IBMBB

It is with great pleasure that I write a few words to mark the occasion of the 4th Annual Scientific Sessions of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo. IBMBB, the only state Institute in Sri Lanka dedicated to postgraduate training and research in Molecular Life Sciences was established with a soft loan from the Swedish Government in 2004. This was the culmination of a SAREC supported research programme led by its Founder Director, Professor Eric H Karunanayake. Once the IBMBB was established, SAREC supported research programme together with its MPhil and PhD trainees were relocated to the IBMBB from the Faculty of Medicine, University of Colombo. Since then several other MPhil and PhD students have registered at the IBMBB, all of them supported either by the SAREC Grant or by other competitive research funds secured by academics of the IBMBB. The first molecular genetics study on breast cancer in Sri Lanka and the first molecular study on phytoplasma in Sri Lanka were carried out as PhD and MPhil studies at the IBMBB. Amongst the MPhil/PhD students who have completed or continuing their studies are three academic staff members from the University of Jaffna and one academic staff member from the Eastern University. In addition to the students registered at the IBMBB, several other MPhil/PhD students registered at other Higher Educational Institutes also carry out all or part of their research work at the IBMBB. Two probationary lecturers, one from the Faculty of Medicine, University of Kelaniya and the other from the Faculty of Medical Sciences, University of Sri Jayawardenapura are among such students. Thus the IBMBB is providing the higher educational sector with much needed academics with extensive research training.

IBMBB commenced two MSc programmes in 2005, one in Molecular Life Sciences and the other in Celluar and Molecular Immunology. Both MSc courses boast a more than 80% completion rate and to date more than 40 students have graduated.

The Annual Scientific Sessions organized by the IBMBB provides a forum for Dissemination of research findings of IBMBB students and postgraduates from other Higher Educational Institutes. In 2009 and 2010 IBMBB did not hold Annual Scientific Sessions as it organized an International Conference in September 2009 to mark IBMBB's 5th Anniversary. Students present their findings at this International Conference and other conferences both local and International in 2009 and 2010. Besides in 2010, IBMBB students also won Four International Awards comprising of two under 34 awards to attend the 14th World Congress on Gynecological

Endocrinology, one of the three best poster awards at the 14th World Congress on Gynecological Endocrinology and SABC Scholarship to attend the San Antonio Breast Cancer Conference, Texas, USA.

During the 4th Annual Scientific Sessions, Professor Stanley Wijesundera Memorial Lecture on "***Drugs from Nature: Harnessing Chemical Diversity in Biodiversity***" will be delivered by Professor E. Dilip de Silva, Senior Professor of Organic Chemistry, Department of Chemistry, University of Colombo. Dr. Erik Bongcam-Rudloff of the Swedish Agricultural University and former chairman of the European Molecular Biology Network will deliver a plenary lecture on "**Next Generation Sequencing".** Research Sessions comprising of 10 oral and 16 poster presentations will follow. Three of the oral and one poster presentation are from students/researchers from other Higher Educational Institutes. From the authors and Institutes given in the abstracts, wide collaboration of IBMBB with other Academic Institutes, Health Sector and Agricultural Sector is clearly evident.

I take this opportunity to thank Vice Chancellor, University of Colombo for her support, Prof De Silva and Dr. Bongcam-Rudloff for agreeing to deliver the Memorial and Plenary Lectures respectively, all our collaborators for facilitating research studies, all those from other Higher Educational Institutes presenting their research at our Scientific Sessions, sponsors for their generous support and, the students and staff of the IBMBB for their dedication and hard work.

Professor Kamani H Tennekoon
Director / IBMBB

Oral Communications

Cancer Genetics, Human DNA Variation

13.45 – 14.00 h (Abstract 01): Novel sequence variants and common recurrent polymorphisms of *BRCA2* in a group of breast cancer patients in Sri Lanka

De Silva S, Tennekoon KH , Karunanayake EH , De Silva JWN, Amarasinghe I, Angunawala P

14.00 – 14.15 h (Abstract 02): A study of genetic polymorphisms in mitochondrial DNA hypervariable regions I and II of the Sri Lankan population.

Ranasinghe RACR, Tennekoon KH, Karunanayake EH, Allen M, Sheriff R, Sheriff MHR,Wijayawardhana H

Reproduction

14.15 – 14.30 h (Abstract 03): Association of 2992 C/T polymorphism in *H19* gene with cord blood levels of insulin-like growth factor (IGF)-II and birth size.

Jayanthiny P, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundere APDeS

14.30 – 14.45 h (Abstract 04): Presence of metalloestrogens in ectopic endometrial tissue

Silva N, Senanayake H, Peiris-John R, Wickremasinghe R, Waduge V

Parasite and Vector Molecular Biology

14.45 – 15.00 h (Abstract 05): Chaperone mediated over expression of parasitic nematod specific growth factor like protein of *Setaria digitata*

Rodrigo WWP, Dassanayake RS, Weerasena OVDSJ, Karunanayake EH

15.00 – 15.15 h (Abstract 06): Molecular characterization of the D3 domain of the 28S ribosomal DNA of the members of the *Phlebotomus argentipes* complex in Sri Lanka.

Gajapathy K, Singh OP, Dykes CL, Adak T, Surendran SN

Plant Molecular Biology

15.15 – 15.30 h (Abstract 07): Revealing Genomic diversity of Sri Lankan traditional, improved and wild rice species.
Gowri R, Weerasena OVDSJ, Fernado KKS

Medicinal Plants

15.30 – 15.45 h (Abstract 08): Effects of a standardized preparation of a decoction comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots), and *Smilax glabra* (rhizome) on the expression of key genes that are involved in tumour suppression, cell cycle arrest and apoptosis in human hepatoma (HepG2) cells
Samarakoon SR, Thabrew I, Galhena PB , Tennekoon KH, de Silva ED

15.45 – 16.00 h (Abstract 09): *In-vitro* protective role of a polyherbal preparation comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome) against bleomycin induced cytogenetic damages in human peripheral blood lymphocytes: A preliminary study
Galhena BP, Thabrew MI, Samarakoon SSR, Solomon FDP, Venkatachalam P, Tennekoon KH

16.00 – 16.15 h (Abstract 10): Anti-inflammatory activity of dried flower extracts of *Aegle marmelos* in Wistar rats
Kumari KDKP, Handunnetti SM , Suresh TS, Samarasinghe K

Poster presentations

Reproduction

PP 01 (Abstract 11): Cord blood leptin levels in newborns from normal uncomplicated singleton pregnancies: Effect of gender and parity.
Karunanayake GA, Tennekoon KH, Jayosoma AA, Jayanthiny P, Karunanayake EH, Kumarasiri JW, Wijesundere APDeS

PP 02 (Abstract 12): A preliminary report on 1737 A/G Polymorphism in *H19* gene in a Sri Lankan cohort of mother-baby pairs
Hewage AS, Jayanthiny P, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundere APDeS

PP 03 (Abstract 13): The CA promoter polymorphism of *IGF1* Gene in pre-eclampsia/ pregnancy induced hypertension: A preliminary report
Yasasni HDP, Jayanthiny P, Sugathadasa BHRK, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDeS

PP 04 (Abstract 14): *H19* polymorphism in newborns born to mothers with pregnancy induced hypertension/ pre-eclampsia: A preliminary report
Sinnathamby S, Jayanthiny P, Sugathadasa BHRK, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDeS

PP 05 (Abstract 15): Placental growth factor levels in normal pregnancy, gestational diabetes mellitus and pregnancy induced hypertension: A preliminary report
Karunanayake GA, Jayanthiny P, Silva NY, Tennekoon KH, Karunanayake EH, Senanayake L

Cancer Biology

PP 06 (Abstract 16): Detection of micrometastasis of breast cancer using cytokeratine-19 based reverse transcriptase polymerase chain reaction.

De Silva WHYD, Pathirana A, Weerasinghe A, Weerasena OVDSJ, Handunnetti SM

Tropical Diseases

PP 07 (Abstract 17): Studies on serum NO_x levels in severe leptospirosis patients in Sri Lanka

Kalugalage TL, Vithanage TDP, Somaratne P, De Silva HJ, Rajapakse S, Handunnetti SM

PP 08 (Abstract 18): Preliminary characterization of some cDNA clones of *Setaria digitata*

Karunanayake GA, Samarakoon SR, Perera ME, Muruganathan A, Tennekoon KH, Karunanayake EH

Plant Molecular Biology

PP 09 (Abstract 19): Genetic Diversity Assessment of Finger millet using microsatellite markers

Kannangara UM, Weeresena OVDSJ, Dasanayake PN

PP 10 (Abstract 20): Isolation and characterization of putative plant disease resistance gene analogues from Sri Lankan rice varieties

Aluwihare WBWMRYC, Weerasena OVDSJ

PP 11 (Abstract 21): Restriction Fragment Length Polymorphism Analysis of the *OSA3* Gene in *Oryza sativa*

Kumarasivam K, Gunawardana D

PP 12 (Abstract 22): Molecular analysis of the F_1 hybrid plants (triploids, ABC) to confirm the true hybridity between *Brassica juncea* and *Brassica oleracea*.

Wickramasekera S, Weerakoon SR, Weerasena OVDSJ

Medicinal Plants

PP 13 (Abstract 23): Effect of *Carica papaya* leaf concentrate on rat platelet count

Gammulle AS, Ratnasooriya WD, Randeniya PV

PP 14 (Abstract 24): Inhibition of intracellular killing mechanisms of human neutroph and the longevity of bioactivities of aqueous leaf extract of *Vitex negundo*.

Sirisena PDNN, Wickremesinghe NKRD, De Silva ED, Ratnasooriya WD, Handunnet SM

PP 15 (Abstract 25): Survivin gene expression in hepatocellular carcinoma cell li optimization of PCR conditions and effect of a herbal decoction

Ratnayaka ARMNNK, Samarakoon SR, Thabrew MI

Proteomics

PP 16 (Abstract 26): Study of IQ motifs of IQGAP2 and IQGAP3 and their interacti with calmodulin

Pathmanathan S, Atcheson E, Hamilton E, Greer B, Harriott P, Timson DJ

Professor Stanley Wijesundera Memorial Lecture

Professor Stanley Wijesundera Memorial Lecture is an annual event of the IBMBB held to commemorate late Professor Stanley Wijesundera, who served as Professor of Biochemistry and as First Vice Chancellor, University of Colombo from 1978-1987. He was a visionary leader during whose tenure infrastructure development and academic progress in the University of Colombo was at its peak. He played a key role in the development of the discipline of Molecular Biology in the University of Colombo. Previous Professor Stanley Wijesundera Memorial Lectures were delivered by Prof. Rune Liminga, Former Director, International Programme in Chemical Sciences, University of Uppsala; Prof. W. D. Lakshman, Former Vice Chancellor, University of Colombo; Prof. Eric Karunanayake, Founder Director, IBMBB and Emeritus Professor of Biochemistry, University of Colombo and Prof. Lal Chandrasena, Senior Professor of Biochemistry, University of Kelaniya. This year Prof Stanley Wijesundera Memorial Lecture will be delivered by Prof E Dilip De Silva, Senior Professor of Organic Chemistry, University of Colombo.

Drugs from Nature: Harnessing Chemical Diversity in Biodiversity

E. Dilip de Silva
Department of Chemistry
University of Colombo

Modern physicians are equipped with a formidable array of clinically useful drugs to treat a large number of diseases that affect humans. From a chemists viewpoint drugs are organic molecules with diverse structures and based on their origins can be categorized into two groups. The first group is made up of synthetic molecules designed and made by chemists in the laboratory while natural products or secondary metabolites made by living organisms and their derivatives constitute the second group. Despite the success of these chemotherapeutic agents there is a pressing need for the development of new pharmaceuticals. Earth's biodiversity is enormous and represent a largely untapped resource for the discovery of lead compounds in the drug discovery process. While terrestrial plants and microorganisms have made major contributions in the past, natural products chemists in their pursuit of finding leads for new pharmaceuticals have ventured in to investigating new areas. Marine ecosystems and endophytic fungi represent two such promising areas for the discovery of new biologically active metabolites. The author's involvement in investigating marine invertebrates, terrestrial plants and fungi for the discovery of new natural products with useful biological activities will be described.

Plenary Lecture: New opportunities created by Next Generation Sequencing Data Analysis technologies

Dr Erik Bongcam-Rudloff
Swedish University of Agricultural Sciences,
University of Uppsala, Sweden

Life sciences have undergone an immense transformation during the recent years, where advances in genomics, proteomics and other high-throughput techniques produce floods of raw data that need to be stored, analysed and interpreted in various ways.

NGS technology massively parallelises nucleotide sequencing procedures, making the sequencing of genomes and of transcriptomes much faster and cheaper than ever before. The new technology is, however, posing massive (bio-) informatics challenges which require new ways of thinking and novel solutions.

During my talk I will present new technologies and briefly discuss the pros and cons of this development. At last I will present exiting new opportunities that this transformation in research strategies generates.

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Abstract No: 01

Novel sequence variants and common recurrent polymorphisms of *BRCA2* in a group of breast cancer patients in Sri Lanka

De Silva S [1], Tennekoon KH [1], Karunanayake EH [1], De Silva JWN[1], Amarasinghe I [2], Angunawala P [3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, 90, Cumaratunga Munidasa Mawatha, Colombo 03, [2] National Cancer Institute, Maharagama, [3] Department of Pathology, Faculty of Medicine, Kynsey Road, Colombo 08, University of Colombo

We have previously reported *BRCA2* mutations and sequence variants on exons 2, 9, 10 and 14 in Sri Lankan breast cancer patients. Here we report mutations and sequence variants in exon 11 of the *BRCA2* gene in the same cohort. There were 55 familial breast cancer patients, 20 at risk individuals and 20 healthy controls. Direct sequencing of exon 11 of *BRCA2* gene showed ten mutations and sequence variants. Mutations observed in exon 11 were c.2403 insA, c.2667 insT, c.5695 A>C, c. 3538 A>C. Of these c.2403 insA and c.2667 insT were novel pathogenic frame-shift additions resulting in a premature stop codon. Five reported silent mutations: c.2457 T>C, c.3624 A>G, c.4035 T>C, c.4791 A>G, c.6741 G>C and one novel silent mutation: c.2766 A>C were also detected in exon11. c.2403 insA was found in 4 unrelated patients and c.2667 insT was found in one patient. c.5695 A>C is a novel possibly pathogenic mutation found in 6 related at risk individuals whereas c. 3538 A>C is a previously reported missense mutation found in one at risk individual. c.2403 insA pathogenic mutation appears to be common among Sri Lanka familial breast cancer patients.

Supported by Sida/ Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of the PhD studies of SDeS.

Abstract No: 02

A study of genetic polymorphisms in mitochondrial DNA hypervariable regions I and II of the Sri Lankan population.

Ranasinghe RACR[1], Tennekoon KH[1], Karunanayake EH[1], Allen M[2], Sheriff R[3], Sheriff MHR[3], Wijayawardhana H[4]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo, [2]Institute of Genetics and Pathology, University of Uppsala, [3]Western Infirmary, Colombo 8, [4]Unuversity of Colombo School of Computing

Analysis of the genetic variations in mitochondrial DNA Hypervariable (HV) regions provides unique information about population diversity. We have previously reported HVI and HVII polymorphisms of the mitochondrial genome in major ethnic groups in Sri Lanka. A haplotype analysis was carried out using our previously reported data and additional samples from the same ethnic groups as well as individuals from Dambana and Hennanigala areas with probable Vedda maternal lineage. A total of 178 subjects (Sinhalese N= 60, Muslims=24, Sri Lankan Tamil N=26, Malay N=31, .Indian Tamil N=5 and probable Vedda group N=32) were studied. Based on HVI and HVII polymorphisms 152 haplotypes were identified. Majority of the haplotypes were seen only in one individual. Among the haplotypes shared between more than one individual indicating same maternal lineage, two were shared between Sinhalese, Muslim and Malay individuals, one was shared between Sinhalese and Muslim individuals, and another was shared between Sinhalese and Malay individuals. Some haplotypes identified within each ethnic group were seen in more than one individual. There were four such haplotypes in the probable Vedda group, two haplotypes each in Malay and Sinhalese individuals and one haplotype each in Muslim and in Sri Lankan Tamil individuals. Thus the probable Vedda group and Sri Lankan Tamils appear to have distinct maternal lineages and some of the Muslim and Malay individuals appear to share a maternal lineage with Sinhalese.

Supported by National Research Council Grant (09-20) and Sida/Secretariat for research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of the PhD studies of RACRR.

Abstract No: 03

Association of 2992 C/T polymorphism in *H19* gene with cord blood levels of insulin-like growth factor (IGF)-II and birth size

Jayanthiny P[1], Tennekoon KH[1], Karunanayake EH[1], Kumarasiri JM[2], Wijesundere APDeS[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Castle Street Hospital for Women, Colombo-8, Sri Lanka

We have previously described an association of *IGF-I* genotype with birth weight. *H19* is a growth regulatory gene expressed exclusively from the maternal allele. *H19* RNA is thought to regulate *IGF-II* transcription. A genetic variation of *H19* was shown to affect birth size in a study from the United Kingdom, but its effect on Sri Lankan or other South Asian populations has not been studied.

The effect of *H19* 2992 C/T polymorphism on cord blood IGF-II levels and birth indices were studied in a cohort of normal healthy newborns (N=60) and their parents (N=60). *H19* polymorphism was genotyped by PCR-RFLP and cord blood IGF-II levels were measured by ELISA. Deviation of genotype frequencies from the Hardy-Weinberg Equilibrium was tested using Chi Square test. Effect of maternal, paternal and newborn *H19* genotypes on cord blood IGF-II levels and birth indices was analysed using two-way ANOVA.

The commonest allele and genotype for the *H19* 2992 C/T polymorphisms were C and C/T respectively. Allele and genotype frequencies did not deviate from Hardy-Weinberg equilibrium. Birth size was affected by the *H19* genotype ($p<0.001$) with the maternal T allele conferring a higher birth weight (One way ANOVA $p<0.05$). Cord blood IGF-II levels did not significantly differ between the three genotypes CC, CT and TT.

Thus, *H19* 2992C/T polymorphism in mothers exerts a significant effect on birth size of Sri Lankan newborns. To refute or confirm this observation the present investigation should be expanded to include a larger number of subjects.

Supported by National Research Council Grant 05-28 and constitutes part of the PhD studies of JP.

Abstract No: 04

Presence of metalloestrogens in ectopic endometrial tissue

Silva N[1], Senanayake H[2], Peiris-John R[1,*], Wickremasinghe R[3], Waduge V[5]

[1]Faculty of Medical Sciences University of Sri Jayewardenepura, [2]Faculty of Medicine, University of Colombo, [3]Faculty of Medicine, University of Kelaniya, [4]Life Sciences Division, Atomic Energy Authority, Colombo

Metalloestrogens, like cadmium, nickel and lead which activate the oestrogen receptors to exert oestrogenic effects are implicated in the aetiology of endometriosis; an oestrogen dependent disease. A case-control study was conducted in a tertiary care hospital to determine the association between metalloestrogens and endometriosis. Fifty cases of endometriosis were compared with 50 age matched controls confirmed by laparoscopy or laparotomy. Blood samples and ectopic endometrial tissue samples were obtained and digested with supra pure 65% HNO_3. Samples were analyzed for cadmium by graphite furnace atomic absorption spectroscopy (GFASS). In a subset of cases (n=30) and controls (n=40) samples were analyzed for nickel and lead by Total Reflection X-ray Fluorescence(TXRF). T-tests and Spearman's correlation coefficient were used for analysis.

Cases had significantly higher (p=0.011) mean(±SD) blood nickel levels(1.69±1.18 µg/L) compared to controls (0.96±0.94 µg/L). Blood levels of cadmium (0.75±0.46 µg/L vs 0.77 ±0.37 µg/L) and lead (5.88±1.25 µg/L vs 3.45±1.25 µg/L) were similar in the two group. Tissue samples had significantly higher (p=0.001, p<0.001, p=0.049 respectively) mean(±SD) levels of cadmium (2.86±0.61 µg/L vs 0.8±0.48 µg/L) nickel (8.64±0.4.12 µg/Lvs1.58±1.21µg/L) and lead (11.22±4.67 µg/L vs 5.86±3.38 µg/L) compared to blood. There was no correlation between blood and tissue levels of cadmium, nickel or lead. In the ectopic endometrial tissue positive correlations were noted between the concentrations of nickel and cadmium (r=0.391, p=0.033), nickel and lead (r=0.566, p=0.001), and cadmium and lead (r=0.421, p=0.016). In conclusion, cadmium, nickel and lead accumulate in the ectopic endometrial tissue.

Supported by University of Sri Jayewardenepura (grant ASP/6/Re/2008/06), ITREOH(NIH grant-Two5497-07) and National Coordinating Committee for Reproductive Health Research and constitutes part of the PhD studies of NS.

*Present Address: Division of Epidemiology and Biostatistics, School of Population Health, Faculty of Medical and Health Sciences, University of Auckland

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- Freeze dryers
- Test Chambers

Abstract No: 05

Chaperone mediated over expression of parasitic nematode specific growth factor like protein of *Setaria digitata*

Rodrigo WWP[1,2], Dassanayake RS[2], Weerasena OVDSJ[1], Karunanayake EH[1]
[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo,
[2]Department of Chemistry, Faculty of Science, University of Colombo.

Setaria digitata is a cattle filarial parasite closely resembles *Wucheraria bancrofti* in many ways and can be used as a model organism to study filarial parasite-specific genes. After analyses of nearly 250 Expressed Sequence Tags (ESTs) of *Setaria digitata,* a cDNA clone containing Open Reading Frame (ORF) of 205 amino acids was identified and of which bioinformatic analyses indicated, the latter ORF to be a parasitic nematode-specific and growth factor like protein. To express this ORF, it was sub-cloned into pET-45b(+) vector making in-frame fusion with the vector derived 6X Histidine residues and this construct then transformed into BL21(DE3) bacterial cells to produce His-tagged recombinant protein. SDS-PAGE analyses of the recombinant proteins following induction with IPTG indicated a little protein is in the soluble fraction and it is largely concentrate into the inclusion bodies. The expression of this protein in BL21(DE3) bacterial cells was confirmed by western blotting with anti-His tag antibody which recognized a ~23kDa protein band on the nitrocellulose membrane.

Co-expression of target protein was carried out with co-transformation of five types of "chaperone team" plasmids (pG-KJE8, pGro7, pKJE7, pG-Tf2 and pTf16). Co-expression of the protein with chaperones showed significant increase of the protein in soluble fraction in SDS-PAGE analyses and of these, the chaperone expressed by plasmid pGro7 shown to produce the highest amount of recombinant protein in soluble fraction.

Supported by National Science Foundation (SIDA/2006/BT/04) and *Sida/Secretariat for research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of the PhD studies of WWPR*

Abstract No: 06

Molecular characterization of the D3 domain of the 28S ribosomal DNA of the members of the *Phlebotomus argentipes* complex in Sri Lanka.

K Gajapathy[1], OP Singh[2], CL Dykes[2] , T Adak[2] and SN Surendran[1]
[1] Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka,
[2]National Institute of Malaria Research, Sector 8, Dwarka, Delhi-110077, India

Phlebotomus argentipes sensu lato (s.l.) Annandale & Brunetti is suspected to be the vector of cutaneous leishmaniasis in Sri Lanka. It exists as a species complex. The Indian *Ph. argentipes* s.l. complex consists of three species namely, *Phlebotomus argentipes* s.s., *Ph. annandalei* Sinton 1923 and *Ph. glaucus* Mitra & Roy 1953. They are morphologically and biologically distinct species. We have tried to distinguish the three species based on the sequence of the D3 domain of the 28S ribosomal DNA (rDNA) to distinguish the members of the complex. DNA was extracted from individual sandflies by method described by Coen *et al.* (1982). The amplified portion of the 28S rDNA using the primers (D3 forward; D3A- 5' GAC CCG TCT TGA AAC ACG GA 3' and reverse; D3B - 5' TCG GAA GGA ACC AGC TAC TA 3') was sequenced. The D3 domain of the 28SrDNA among the members of the *Phlebotomus argentipes* species complex shows no variation.

Supported by the University Grants Commission and National Research Council of Sri Lanka and constitutes part of PhD studies of KG.

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Abstract No: 07

Revealing Genomic diversity of Sri Lankan traditional, improved and wild rice species.

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[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo. [2]Agricultural Biotechnology Centre, University of Peradeniya

Sri Lanka has a valuable repository of germplasm collection due to the availability of a large number of different traditional and improved rice varieties. However, no proper studies have been carried out on genetic diversity of those varieties. Amplified Fragment Length polymorphism (AFLP) was used to evaluate the genetic diversity among rice varieties available in the germplasm collection of Plant Genetic Resources Centre, Sri Lanka. AFLP analysis of rice varieties using ten different primer combinations yielded 98.4% of polymorphism for traditional rice varieties and 92.4% polymorphism for improved varieties. Genetic similarities were estimated using Jaccard's (*J*) similarity coefficient. Unweighted Pair Group Method with arithmetic mean (UPGMA) based dendrograms were constructed. The dendrograms separated accessions into clusters. Principal Component analysis further confirmed the patterns obtained by the cluster analysis. The high values of Cophenetic correlation strongly supported the clustering pattern of UPGMA dendrograms. Rice accessions in the same cluster showed similar morphological characteristics (E.g. height, grain colour etc) while the accessions which are known to be morphologically and genetically distinct got separated from others. The outliers used in this analysis uniquely separated and all methods of analysis produced similar results, confirming the reliability of data used and analysis of this study. This genetic diversity data will provide more direct and reliable genetic information for selecting suitable parents in rice breeding programs. Furthermore, the information will be useful in management of *in situ* and *ex situ* preservations of rice germplasm.

Supported by National Research Council of Sri Lanka (Grant no 05/61) and constitutes part of PhD studies of GR

Abstract No: 08

Effects of a standardized preparation of a decoction comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots), and *Smilax glabra* (rhizome) on the expression of key genes that are involved in tumour suppression, cell cycle arrest and apoptosis in human hepatoma (HepG2) cells

Samarakoon SR[1], Thabrew I[1], Galhena PB[2], Tennekoon KH[1], de Silva ED[3]

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A decoction of *Nigella sativa* seeds, *Hemidesmus indicus* roots and *Smilax glabra* rhizomes used as a traditional medicine for cancer was found to protect against chemically induced hepatocarcinogenesis in the rat. Recently we have shown that the anti-hepatocarcinogenic activity is at least partly mediated by selective cytotoxicity, antioxidant activity and anti-inflammatory activity. However, the underlying molecular mechanisms responsible for the anti-hepatocarcinogenic action are not known. We evaluated the effect of standardized decoction on the expression of tumour suppressor, cell cycle control, apoptic and ani-apoptic genes (*p53* and *p21*; *NfkB p50* and *p65*; *IKK-*□, *Bax* and *Bcl-2*) on human hepatoma (HepG2) cells with the long term goal of developing the above herbal formulation into a form that may be used by itself or as an adjunct to existing therapies for the treatment of hepatocellular carcinoma.

The effects of the decoction on (a) mRNA and (b) protein expression of the selected genes were evaluated by (a) reverse transcription PCR and (b) immunohistochemical and/or western blot analysis. The results demonstrated that the standardized decoction can significantly ($p<0.01$) enhance the expression of *p53*, *p21* and *Bax* genes while reducing the expression of *IKK-*□and *Bcl-2* genes in HepG2 cells. Immunohistochemical analysis confirmed the reduction of nuclear translocation of NfkB *p50* protein in HepG2 cells treated with the decoction. Overall results obtained demonstrate that the decoction may mediate its reported anti-hepatocarcinogenic effects at least in part, through modulating activities of genes involved in tumour suppression, cell cycle arrest and apoptosis.

Supported by National Science Foundation (NSF), Sri Lanka and Sida/Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of PhD studies of SRS

Abstract No: 09

In-vitro protective role of a polyherbal preparation comprised of _Nigella sativa_ (seeds), _Hemidesmus indicus_ (roots) and _Smilax glabra_ (rhizome) against bleomycin induced cytogenetic damages in human peripheral blood lymphocytes: A preliminary study

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Cytogenetic damages in peripheral blood lymphocytes (PBL) mediated by bleomycin, a frequently used chemotherapeutic drug is thought to be mediated by DNA double strand breaks (DSB) and restricts its clinical use. Quantification of DSB in the nucleus by targeting the functions of natural proteins at the site of DSB repair has emerged recently as a sensitive index to monitor cytogenetic damages.

Recent *in-vitro* and *in-vivo* studies have demonstrated the presence of potent free radical scavenging activity in the polyherbal preparation consisting of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome). Therefore, the present study was carried out to investigate any possible protective role of the above polyherbal preparation against bleomycin induced DSB in PBLs.

Isolated PBLs were exposed to bleomycin at a dose of 40µg / ml in the presence or absence of the decoction (100µg / ml). Modulatory effect of the decoction on bleomycin induced DSB was evaluated by fluorescent staining of the phosphorylated chromatin H2AX by incubating cells with anti-γH2AX antibody (clone JBW301, Upstate, Charlottesville, VA).

A significant reduction ($p < 0.05$) in γ H2AX foci was observed in PBL exposed to bleomycin in the presence of the decoction when compared with the cells exposed to bleomycin only. γH2AX foci in PBL exposed to decoction only, was similar to that of untreated PBL, thus indicating absence of cytogenetic damages by the decoction itself. These findings indicate a protective effect of the test decoction against cytogenetic damage induced by bleomycin in human PBLs.

Supported by India – Sri Lanka Foundation for collaborative research and constitutes part of PhD studies of BPG.

Abstract No: 10

Anti-inflammatory activity of dried flower extracts of *Aegle marmelos* in Wistar rats

Kumari KDKP [1], Handunnetti SM [2], Suresh TS [1] and Samarasinghe K [3]
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Various parts of *Aegle marmelos* (AM) are widely used in traditional medicine for the treatment of different disorders. As reported by Sujeewani and Suresh (2009), the flower extract exhibited DPPH scavenging activity and it is capable of reducing the oxidative stress in Wistar rats. Therefore the present study was designed to study the anti-inflammatory effect of dried flower extracts of *Aegle marmelos* in healthy and diabetic rats.

Rats were randomly divided into 6 groups (n=6) and 2.5 ml each of following were administered one hour before inducing paw oedema. Distilled water, Indomethacin (10 mg/kg), ethanol extract (25 mg/kg), water extract (100, 200, 400 mg/kg) were given to groups 1-6 respectively. The baseline values of paw volume taken at the zero hour. Hind paw oedema was induced by injecting 0.01ml of carrageenan (1%) and paw volumes were measured at 1, 2, 3, 4 and 5 hr using a plethysmometer.

Water and ethanol extracts of dried flowers of AM showed statistically significant ($p<0.05$) inhibition of paw oedema compared to control group. The dose of 200 mg/kg of water extract exhibited maximum inhibition of 69.9% at 2 hr. The dose of 400 mg/kg showed highest inhibition (61.42%) at 5hr. The ethanol extract gave 67.32% and 64.49% inhibitions at 2 and 3hr respectively. Indomethacin significantly decreased paw oedema with highest inhibition level of 66.01% at 2 hr. *Aegle marmelos* dried flower extracts showed a statistically significant acute anti-inflammatory activity in the carrageenan-induced rat paw oedema model. Both extracts exerted anti inflammatory effects comparable with indomethacin.

Supported by University of Sri Jayewardenepura (ASP/06/R/2009/10).

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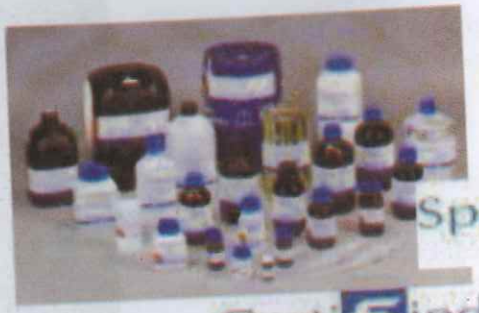

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Estd. 1841

Abstract No: 11

Cord blood leptin levels in newborns from normal uncomplicated singleton pregnancies: Effect of gender and parity

Karunanayake GA[1], Tennekoon KH[1], Jayosoma AA[1], Jayanthiny P[1], Karunanayake EH[1], Kumarasiri JW[2], Wijesundere APDeS[2]

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Leptin a hormone secreted from adipose tissue is also produced by the placenta. To examine the effect of gender and parity on leptin levels, cord blood leptin concentrations were measured in 110 newborns (female=50, male =60) using enzyme-linked immunosorbent assay. Cord blood leptin levels were [geometric mean (95% Cl)] 8.512 (7.181, 10.09) ng/ml. When stratified by gender and parity first born males [N = 32; geometric mean (95% Cl): 5.699 (4.009, 8.102) ng/ml] had significantly lower levels of cord blood leptin compared to second born males [N = 28; 9.861 (7.187, 13.53) ng/ml] and females [first born: N = 24; 10.78 (7.594, 15.31) ng/ml, second born: N = 26; 9.568 (6.838, 13.39) ng/ml]. Leptin levels normalized to ponderal index remained significantly (P = 0.018) low in fist born males compared to first born females. Although leptin normalized to ponderal index in first born males were also lower than in second born males the difference was statistically not significant. Cord blood leptin concentrations were significantly and positively correlated with birth weight (r = 0.3903, P<0.0001), crown heel length (r = 0.2633, P = 0.0054), head circumference (r = 0.3191, P = 0.0007), and ponderal index (r = 0.2187, P = 0.0217). When stratified by gender and parity these correlations did not persist in second born males. Significant correlations were not seen with crown heel length in second born females and head circumference in first born females. Thus both parity and gender appear to modulate cord blood leptin level and its association with birth indices.

Supported by National Research Council Grant (NRC 05-28)

Abstract No: 12

A preliminary report on 1737 A/G Polymorphism in *H19* gene in a Sri Lankan cohort of mother-baby pairs

Hewage AS[1], Jayanthiny P[1], Tennekoon KH[1], Karunanayake EH[1], Kumarasiri JM[2], Wijesundere APDeS[2]

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Common genetic variations of imprinted genes that are maternally expressed could elucidate the maternal-specific inheritance of low birth weight. More than 20 million infants worldwide are born with low birth weight every year and are at risk of having serious developmental problems and increased risk of non-communicable diseases in later life. Human *H19* is an imprinted gene located on chromosome 11p15.5. It is exclusively expressed from the maternal allele and code for a regulatory RNA implicated in the regulation of insulin-like growth factor II. A previous study from United Kingdom which investigated three common Single Nucleotide Polymorphisms (SNPs) in the *H19* gene 2992C/T, 1737A/G and 3228A/G found an association between 2992C/T polymorphism and birth size. *H19* 1737A/G SNP and its effect on birth size have not been previously studied in Sri Lanka. We studied *H19* 1737A/G genotype frequency in mother-baby pairs (N=24), comprising of healthy mothers having uncomplicated singleton term pregnancies and babies born were apparently healthy. *H19* 1737A/G genotype was determined by PCR based RFLP. Distribution of genotypes AA, AG and GG were 0%, 33%, 66% for mothers and 0%, 25%, 75% for newborns. The allele frequencies of A and G were 0.16 and 0.83 for mothers, 0.125 and 0.875 for newborns. GG homozygosity was more frequent in this sample when compared to frequencies reported from United Kingdom. Further studies are in progress to include a larger sample and to determine the association of genotype frequencies and birth indices between different genotypes.

Supported by NRC Grant 05-28 and Sida/Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of MPhil studies of ASH

Abstract No: 13

The CA Promoter Polymorphism of *IGF1* Gene in Pre-eclampsia/ (PIH) Pregnancy Induced Hypertension

Yasasni HDP[1], Jayanthiny P[1], Sugathadasa BHRK[1], Tennekoon KH[1], Karunanayake EH[1], Kumarasiri JM[2], Wijesundera APDeS[2]

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Pre-eclampsia / pregnancy induced hypertension (PIH) is a polygenic disorder affecting approximately 5% of pregnant women. Polymorphisms in several genes are implicated in its aetiology. Lower levels of Insulin-like growth factor-1 (IGF1) have been shown to be associated with pre-eclampsia / (PIH). *IGF1* gene polymorphism in the promoter region of the *IGF1* gene may directly influence the expression of *IGF1*. Polymorphisms of the *IGF1* gene and, in particular, a common *IGF1* promoter CA repeat, has been reported to be associated with size at birth and IGF-I levels. There are no reports on the CA promoter polymorphism of *IGF1* gene in pre-eclampsia/ (PIH). The objective of this study was to describe the CA promoter polymorphism of *IGF1* gene in pre-eclampsia / PIH (N=15) in a cohort of Sri Lankan women. DNA was extracted from peripheral venous blood and polymerase chain reaction was used to amplify the region of the gene containing the promoter CA repeat. PCR products were subjected to fragment analysis using MegaBACE 1000 DNA sequencer. Results were analyzed using the MegaBACE genetic profiler 2.0 software. 198-bp allele was the most frequent (54.2%) and the second commonest allele was the 196-bp allele (33.3%) in this cohort. Prevalence of the 198-bp allele known to be associated with lower IGF-I levels was higher than the prevalence reported from this laboratory for normal pregnancies (38.5%). These findings need to be confirmed on a larger sample of patients.

Supported by the Swedish Agency for Research Cooperation with Developing Countries (SAREC) grant for Molecular Biology and Biotechnology and National Research Council (Grant NRC 05-28) and constituted part of MSc studies of HDPY.

Abstract No: 14

_H19_ polymorphism in newborns born to mothers with pregnancy induced hypertension/ preeclampsia: A preliminary report

Sinnathamby S, Jayanthiny P, Sugathadasa BHRK, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDeS

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H19 gene is an imprinted noncoding gene, and functions exclusively at RNA level. H19 RNA has several functions including regulation of invasion and migration of trophoblast cells. The biological role of *H19* in embryonic cells implicates a significant role for *H19* in the pathogenesis of preeclampsia (PE)/ pregnancy induced hypertension (PIH). Preeclampsia is a rapidly progressing, heterogeneous, multifactorial disease which threatens the life of the mother and offspring. Furthermore, PE/PIH is known to be associated with low birth weight and *H19* 2992 C/T polymorphism is reported to be associated with low birth weight. This is a preliminary study which was designed to investigate the polymorphism in *H19* 2992 C/T polymorphism in a cohort of newborns, born to women with PE/PIH. Genomic DNA was extracted from cord blood samples collected at birth. Polymorphic region was amplified using PCR technique with specific primers, under optimized conditions. The PCR products were purified and subjected to RFLP analysis. Homozygosity for the T allele reported to be associated with higher birth weight was found only in 5 out of 16 samples tested. A larger study including mothers and newborns from PE/PIH pregnancies and normal pregnancies is needed to clarify whether the prevalence of T allele is significantly lower among PE/PIH patients and their newborns.

Supported by the Swedish Agency for Research Cooperation with Developing Countries (SAREC) grant for Molecular Biology and Biotechnology and National Research Council (Grant NRC 05-28) and constituted part of MSc studies of SS.

Abstract No: 15

Placental growth factor levels in normal pregnancy, gestational diabetes mellitus and pregnancy induced hypertension: A preliminary report

Karunanayake GA[1], Tennekoon KH[1], Jayanthiny P[1], Silva NY[1], Karunanayake EH[1], Senanayake L[2]

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Placental growth factor (PLGF) is a member of vascular endothelial growth factor, produced predominantly by the placenta. To compare the maternal blood PLGF concentration between normal pregnancies, gestational diabetes mellitus and pregnancy induced hypertension, serum PLGF levels in maternal blood were measured in normal pregnancies (N = 15), gestational diabetes mellitus (N = 15) and pregnancy induced hypertension (N = 15) using enzyme-linked immunosorbent assay. Birth weights were comparable between all three groups. Maternal blood PLGF levels were [geometric mean (95% Cl)] 107.399 (61.659, 187.499) pg/ml, 62.087 (34.198, 112.979) pg/ml, 132.739 (74.131, 237.684) pg/ml respectively in normal pregnancies, gestational diabetes mellitus and pregnancy induced hypertension. Although maternal blood PLGF concentration in gestational diabetes mellitus were lower when compared to normal pregnancies and pregnancy induced hypertension the difference was statistically not significant (Kruskal-Wallis ANOVA, P = 0.0809). Furthermore maternal blood PLGF levels did not significantly correlate with birth weight in any of the groups. A larger sample needs to be studied to ascertain whether gestational diabetes mellitus results in lower maternal blood PLGF level.

This work was supported by NRC Grant 05-28.

Abstract No: 16

Detection of micrometastasis of breast cancer using cytokeratine-19 based reverse transcriptase polymerase chain reaction

De Silva WHYD[1], Pathirana A[2,3], Weerasinghe A[4], Weerasena OVDSJ[1], Handunnetti SM[1].
[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Colombo South Teaching Hospital, Kalubowila, [3]Dept of Surgery, Faculty of Medical Sciences, University of Sri Jayawardenapura, [4]Faculty of Medicine and Allied Health Sciences, Rajarata University

Breast cancer is the most common cancer in women in developed countries, and it is the most frequent malignancy diagnosed among women in Sri Lanka. The presence of metastases in lymph nodes is the most powerful prognostic factor in breast cancer patients. Routine histological examination of lymph nodes has limited sensitivity for the detection of breast cancer micrometastases. The aim of the present study was to establish and optimize a reverse transcription polymerase chain reaction(RT-PCR) assay for detection of micrometastasis/ minimal residual disease based on cytokeratin-19 mRNA from axillary lymph nodes (ALN) of breast cancer patients. RT-PCR was successfully established with RNA obtained from breast tissue samples of three primary breast cancers (positive control) using primers for cytokeratin (CK-19) and β-actin (controls). An unaffected lymph node sample from a non-breast cancer patient who was undergoing thyroidectomy, and peripheral blood leukocytes of two healthy volunteers served as negative controls. Out of three positive controls, two samples showed strong PCR amplification for CK-19 marker with a 460 bp fragment. None of the 3 negative controls were amplified by CK-19. All control samples (3 positive and 3 negative) were amplified by β-actin indicating the presence of RNA in these samples. Among the 2 ALNs tested, breast cancer metastases were not detected by histopathology, but one ALN gave a CK-19 band by RT–PCR. This preliminary study shows that RT–PCR assay for CK-19 is possibly more sensitive compared to histopathology and can be extended further to a large-scale study of breast cancer patients. The RT-PCR methodology may be further improved with the use of multimarkers and sentinel lymph node samples to increase sensitivity to provide a prognostic indicator for breast cancer.

Supported by IBMBB and constitutes part of MSc studies of WHYDDeS

Abstract No: 17

Studies on serum NO_x levels in severe leptospirosis patients in Sri Lanka

Kalugalage TL[1], Vithanage TDP[2], Somaratne P[3], De Silva HJ[4], Rajapakse S[2], Handunnetti SM[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Clinical Medicine, Faculty of Medicine, University of Colombo, [3]Department of Microbiology, Medical Research Institute, Colombo 08, [4]Department of Medicine, Faculty of Medicine, University of Kelaniya

Leptospirosis is an endemic disease in Sri Lanka caused by genus *Leptospira*. The underlying pathogenic mechanisms associated with severe manifestations have been attributed mainly to endothelial dysfunction and tissue damage mediated by pro-inflammatory cytokines and inflammatory mediators such as nitric oxide ($NO^{\bullet}$). $NO^{\bullet}$ has a short half-life and it converts to nitrite (NO_2^-) and nitrate (NO_3^-), referred to as NOx. In the present study serum NOx levels were compared between severe and mild patients and the possibility of using serum NOx levels as a prognostic marker of disease severity was examined. Patients were categorized according to their *m*icroscopic *a*gglutination *t*est (MAT) titre and disease severity; severe leptospirosis (≥800; n=24), mild leptospirosis (≥800; n=13) and MAT equivocal (100-400; n=30). Non-leptospirosis fever patients (<100; n=18) and healthy controls were used for comparison. Severe patients had significantly higher NOx levels (32.1±12.2μM) compared to mild patients (21.6±6.4μM), MAT equivocal patients (15.2±8.5μM), non-leptospirosis fever (13.5±11.5μM) and healthy controls (5.8±2.5μM) ($P<0.01$). In severe and mid leptospirosis patients, both serum nitrite levels and MAT titres were not significantly different. Mean age between patient groups were comparable. Duration between onset of symptoms and sampling of severe and mild leptospirosis patients and non-leptospirosis fever patients were comparable, but significantly higher compared to MAT equivocal patients ($P=0.036$). In confirmed leptospirosis patients, serum NOx levels were significantly correlated with serum creatinine ($r=0.470$; $P=0.003$), blood urea ($r=0.369$; $P=0.025$) and total bilirubin ($r=0.383$; $P=0.019$). These results indicate that $NO^{\bullet}$ may play an important role in pathogenesis and may be a prognostic indicator of severe leptospirosis.

Supported by IBMBB and Medical Research Institute and constitutes part of MSc studies of TLK.

Abstract No: 18

Preliminary characterization of some cDNA clones of *Setaria digitata*

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Wuchereria bancrofti, the causative agent of lymphatic filariasis in Sri Lanka cannot be cultured. Therefore the cattle filarial parasite, *Setaria digitata* is used as a model organism to study filarial parasite genome. In-vivo excised ten cDNA clones of *S. digitata* (designated as cDNAsd-9 to cDNAsd-19) were selected following screening of a cDNA library constructed in Zap Express system. DNA was extracted, insert sizes estimated by restriction digestion and sequenced using automated MegaBACE 1000 sequencer. Sequences obtained were analysed using Bioinformatics tools. Nucleotide sequence of cDNAsd-9 clone (530 bp) aligned with mRNA sequence of *Loa loa* hypothetical protein with 81 % homology and *Brugia malayi* partial mRNA with 83 % homology. The ORF of cDNAsd-9 encodes a 96 amino acid protein (molecular weight: 11238.94 Da, isoelectric point: 8.60). This predicted protein is neither a mitochondrial nor a secretory protein. The sequence of cDNAsd-12 (904 bp) aligned with the sequence of *B. malayi* cell death abnormality protein 8 partial mRNA with 70 % homology. The ORF of this sequence encodes a 72 amino acid protein and predicted as a trans-membrane protein (molecular weight: 8459.2 Da, isoelectric point: 8.93). cDNAsd-13 clone of 961 bp aligned with *L. loa* hypothetical protein (LOAG_04881) mRNA with 80 % homology. The ORF of cDNAsd-13 encodes 113 amino acids (molecular weight: 12994.91 Da, isoelectric point: 9.51). The predicted protein is neither a trans-membrane nor a secretory protein. Furthermore nucleotide sequence of cDNAsd-16 (775 bp) partially aligned with mRNA sequence of *L. loa* hypothetical protein (LOAG_05978) with 75 % homology.

This work was supported by Sida/Secretariat for research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology.

Abstract No: 19

Genetic Diversity Assessment of Finger millet using microsatellite markers

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Finger millet (*Eleusine coracana*) is a food crop, well known for its nutritional value and health benefits, such as hypoglycemic, hypocholestrolemic and anti-ulcerative properties. Knowledge of genetic diversity has a significant impact on the improvement of crop plants as well as germplasm conservation. Molecular markers provide the best estimate of genetic diversity since they are independent of environmental factors. Characterization of finger millet accessions in Sri Lanka has been done using only morphological markers which are dependent on environmental factors. Although a preliminary study on genetic differentiation of finger millet using AFLP markers has been carried out, more studies using several DNA markers is required to validate the phylogenetic relationships and classification. The main objective of this study was to understand the genetic variation of finger millet germplasm accessions in Sri Lanka using SSR markers. Seeds of 17 finger millet accessions were grown under greenhouse conditions and 2-3 week old leaves were harvested for the extraction of DNA. Three SSR markers, which represented three different linkage groups of the finger millet linkage map were selected for this study. The PCR amplification was carried out using a touchdown program and the amplification products were separated in 6% denaturing polyacrylamide gels and visualized by silver staining. This diversity study using three SSR markers revealed a very low diversity in studied Sri Lankan finger millet accessions. Further studies with more markers and accessions are needed for the understanding of true genetic diversity.

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Abstract No: 20

Isolation and characterization of putative plant disease resistance gene analogues from Sri Lankan rice varieties

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Rice suffers from many important diseases and an analysis of resistance gene analogues offers a means to identify DNA markers linked to resistance loci. This study was carried out to identify and characterize resistance gene analogs (RGAs) in rice by PCR amplification of genomic DNA using primers designed based on the conserved amino-acid domains of several known plant disease resistance related protein sequences. Five wild rice species, weedy rice (*Hygroryza aristata*), two new improved and two traditional rice varieties were used in this study. Fragments amplified by RGA primers were separated by polyacrylamide gel electrophoresis and specific bands were exised and DNA was eluted and re-amplified by using same RGA primers. Re-amplified products were then purified and sequenced. The resulted sequences were analyzed by using bioinformatics analysis tools and data bases.

A fragment amplified from *Hygroryza*, by NLRR primer showed best alignment with the chromosome 10 of *Oryza sativa-Nipponbara* cultivar, (chromosome region 9,685,904 to 9,689,438 with gene LOC-Os10g19160). This gene code for a protein with kinase superfamily-leucin rich repeats, Tyr-pkinase-ser-thr-pkinase and toll-like receptor-LRR domains. These domains are found to be associated with plant disease resistance proteins. Therefore the fragment isolated could be related to disease resistance. However, another fragment amplified from *Oryza granulata*, by NLRR primer (designed on LRR region of tobacco disease resistance gene), showed alignment with a gene in chromosome 6 of *Oryza sativa-japonica* cultivar only with grass Pollen allergen domain. This could be due to the non specific annealing of primers as considerable degeneracy in RGA primers.

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Abstract No: 21

Restriction Fragment Length Polymorphism Analysis of the *OSA3* Gene in *Oryza sativa*

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Soil salinity is a major concern in Sri Lanka in both the coastal belt and in poorly irrigated inland cultivations in the dry zone. Rice, which demonstrates high levels of salinity tolerance in both the germination and vegetative growth stages, can be utilized to reclaim saline soil. Resistance to soil salinity is conferred by multiple mechanisms, which are mediated by the selective up regulation of expression of signaling, transporter, stress-responsive and defense genes. One such gene that is up regulated is the *OSA3* gene that is implicated in Na^+ ion homeostasis. In this study, we have conducted a RFLP analysis of the *OSA3* gene to identify possible polymorphic sites that may contribute to increased salt tolerance. Rice genomic DNA was extracted from seven varieties, which included salt tolerant, salt susceptible and newly improved salt tolerant varieties. A 5' partial fragment of the *OSA3* gene was amplified using PCR from the extracted DNA templates. The PCR products were purified and were subjected to RFLP analyses using the restriction enzymes *Hpa*II, *Hga*I and *Pst*I. Digestion profiles of the *Hpa*II and *Hga*I restriction enzymes did not demonstrate any differences in their fragment patterns. However, the digestion profile of *Pst*I demonstrated distinct fragment patterns for salt tolerant varieties such as Pokkali and Bg11-139 when compared to the salt susceptible ones. The putative polymorphic sites found within the partial fragment of *OSA3* require to be confirmed by sequencing. Future studies centered on the *OSA3* gene should consider other restriction enzyme sites for RFLP analyses.

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Abstract No: 22

Molecular analysis of the F_1 hybrid plants (triploids, ABC) to confirm the true hybridity between *Brassica juncea* and *Brassica oleracea*.

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Polyploidy, is recognized as a major mechanism in plant evolution. There are many successful hexaploids (6x) in crops such as bread wheat, and kiwifruit exist, hexaploid *Brassica* are still not available. *B.juncea* is a tetraploid containing AABB genomes and *B. oleracea* contains only CC genome which is diploid. Hence, a hexaploid *Brassica* (6n, AABBCC) population generated by combining the C genome of the *B. oleracea* and, A and B genomes of *B. juncea* will lead to widen the genetic variability among members of the family *Brassicaceae.* An investigation was conducted to evaluate the possibility of synthesizing trigenomic (ABC) *Brassica* by crossing *Brassica juncea* (L.) Czern & Coss and *B. oleracea* (L.). Five genotypes of *B. juncea* and *B. oleracea* were selected for the study. Simple Sequence Repeats (SSR) microsatellites were used as DNA markers for molecular confirmation of putative hybrids (ABC). Two selected SSR markers, OI10-F09 and sN12353 were used and polymerase chain reaction (PCR) amplification was carried out with genomic DNA of parents and F_1 hybrids. Evaluation of the putative hybrids by molecular markers confirmed seven true hybrids. Although success rate of pod and seed formation of crosses between *B. juncea* and *B. oleracea* was low, production of true interspecific hybrids was successful. The present investigation confirms that hybridization of tetraploid *B. juncea* (4n, AABB) with diploid *B. oleracea* (2n, CC) is a potential approach to produce hexaploid *Brassica* genotypes.

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Abstract No: 23

Effect of *Carica papaya* leaf concentrate on rat platelet count

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This study scientifically investigated the platelet increasing potential of *Carica papaya* leaf concentrate (CLC), in terms of numbers and percentage, in a thrombocytopenia induced rat model, established for the first time, through bone marrow suppression.

Thrombocytopenia was established in 4 groups (N=6/group) of Wistar rats by oral administration of Hydroxyurea (15mg/kg). Three doses (low dose – LD 0.18ml/100g, human equivalent dose – HE 0.36 ml/100g and high dose – HD 0.72 ml/100g)) of freshly prepared CLC were orally administered, once daily for 3 consecutive days to platelet depleted groups, while a normal, non-thrombocytopenic group (N=6) was treated with only LD of CLC. Control groups were treated with distilled water. Platelets of all 6 groups were evaluated daily.

Normal, non-thrombocytopenic rats with LD of CLC increased their platelets by 19.17%. The LD, HE and HD of CLC in thrombocytopenia induced rats increased platelets by 14.27%, 14.28% and 76.5%, respectively, all significantly higher ($P \leq 0.05$) compared to the control.

The CLC was well tolerated by rats over a period of 3 days showing neither overt signs of toxicity (salivation, diarrhea, stress, aversive behavior etc), hepatic (in terms of serum GOT, GPT levels), renal (in terms of serum Creatinine and urea), hematological nor neurological (in terms bar and bridge tests) toxicity.

In conclusion, this study for the first time demonstrated that freshly prepared CLC from mature leaves of *Carica papaya*, is a safe (non-toxic), orally active agent that can effectively increase rat platelets. Thus, the CLC has the potential to be developed as a plant based therapeutic agent for thrombocytopenia.

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Abstract No: 24

Inhibition of intracellular killing mechanisms of human neutrophils and the longevity of bioactivities of aqueous leaf extract of *Vitex negundo*.

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Neutrophils play an important role in the early phase of inflammatory response. Reactive oxygen intermediates (ROI) and reactive nitrogen intermediates produced during phagocytosis contribute to intracellular killing mechanisms of neutrophils. *Vitex negundo* is used in traditional medicine and its anti-inflammatory activity has been scientifically validated previously. Present study investigated the effect of freeze-dried aqueous leaf extract (FLE) of *V. negundo* on neutrophil phagocytic activity, its intracellular killing functions (ROI and nitric oxide (NO) production) and longevity of these bioactivities. Cytotoxicity assay showed suitability of concentrations below 1000 µg/ml for the *in vitro* assays. FLE showed 55% inhibition of yeast phagocytosis, 54% inhibition of ROI production at 500 µg/ml and the inhibitory effects were dose-dependent ($r=0.94$; $P=0.015$ and $r=0.756$; $P=0.007$ respectively). Inhibition of NO production was 100% and 87.5% as assessed by NO_2 and NO_x levels at 500 µg/ml of FLE and both were dose-dependent ($r=0.898$; $P<0.001$ and $r=0.883$; $P< 0.001$ respectively). Comparison of bioactivities in three FLE preparations, prepared prior to 1-2 weeks, 3 months and 9 months showed that ROI and NO inhibitory activity of the two older preps were similar to that of the 1-2 week old Prep (51% and 46 % for ROI and 85 and 83% for NO respectively). Similarly, other aqueous extracts (leaves of *Ixora coccinea* and plants of *Andrographis paniculata)* showed high ROI inhibitory activity when tested after 12 and 25 months. Aqueous leaf extract of *V. negundo* inhibits the inflammatory responses by human neutrophils and could be used for further development of specific anti-inflammatory agents.

This work was supported by NRC Grant 05-52.

Abstract No: 25

Survivin gene expression in hepatocellular carcinoma cell line: optimization of PCR conditions and effect of a herbal decoction

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Survivin, an inhibitor of apoptosis protein is highly expressed in cancers but undetectable in most normal adult tissues. Expression of survivin in cancer cells is reported to be associated with cancer progression and drug resistance. Thus, control of its expression in cancer cells has significant consequences for cancer therapeutics. The present study was carried out to investigate the effect of a decoction prepared from *N. sativa* seeds, *H. indicus* roots and *S. glabra* rhizomes on expression of survivin gene in human hepatocellular carcinoma (HepG2) cells. This decoction has been reported to (a) have potent anti-hepatocarcinogenic activity and (b) exert significant cytotoxicity to HepG2 cells.

To investigate the effect of the decoction on survivin gene expression, HepG2 cells were initially cultured in DMEM medium for 24 h at 37 ^{0}C in 95% air/ 5% CO_2 atmosphere, with 95% humidity. Cells were then treated for a further 24 h with different concentrations of the decoction (150 µg/ml to1200 µg/ml). Control cells were treated with 1 % DMSO in DMEM medium. Total RNA was extracted from cultured cells and expression of survivin was determined by RT-PCR. Expression of survivin in HepG2 cells was significantly (One way ANOVA, $P < 0.05$) downregulated by the decoction in a dose dependant manner. The downregulation of survivin expression at the transcriptional level could be one of the mechanisms by which the anti-carcinogenic effect of the decoction is mediated.

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Abstract No: 26

Study of IQ motifs of IQGAP2 and IQGAP3 and their interactions with calmodulin

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IQGAP-like proteins are found in a number of organisms including yeast and human. The role of IQ motifs in target recognition and signal transduction is immensely complex. Calmodulin is a calcium binding protein which binds to IQ motifs of IQGAP-like proteins. Here we show these interactions using synthetic peptides and native gel electrophoresis. Except for the first and fourth IQ motifs of IQGAP2, all the other IQ motifs of IQGAP2 and IQGAP3 interacted with calmodulin in presence of calcium ions whereas the first IQ motif of both the IQGAPs interacted with apocalmodulin. However a notable feature was that some of the interactions were stable and others were of transient type. These findings extend our knowledge on IQ motif- EF hand protein interactions.

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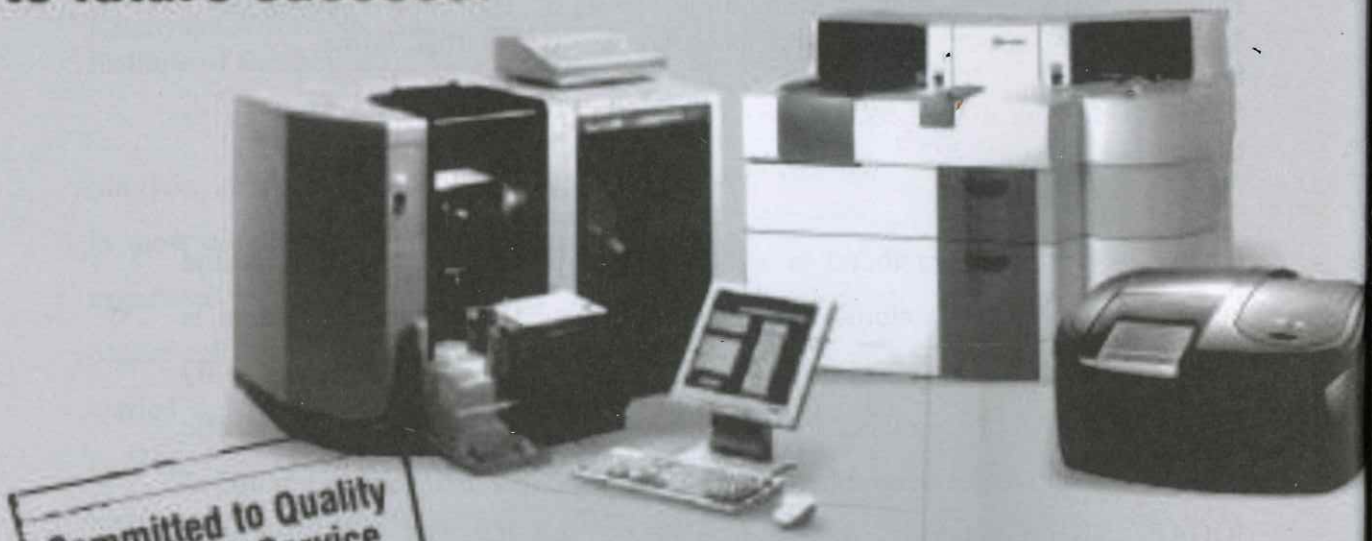

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