

BIOCHEMISTRY RESEARCH DIVISION

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The Biochemistry Research Division is actively involved in research activities of Malaria, Snake bite and Environmental Health.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 MALARIA

1.1.1 Validation of point of care test in detection of Glucose-6-Phosphate Dehydrogenase enzyme deficiency

This study assessed the performances of two qualitative tests, the fluorescent spot test and the CareStart™ test, against the gold standard quantitative Spectrophotometric assay in population of 1000 healthy volunteers living in Ahlone and Insein Township of Yangon Region. Bamar (763), Karen (119), mix ethnicity (32), Rakhine (28), Chin (10) and Talgu (5) ethnic groups were tested in this study. For G6PD fluorescent spot test (Trinity Biotech, Ireland) was used. The spots were then visualized under UV light, spots that showed fluorescence were classified as normal, and spots that failed to show fluorescence were classified as deficient. The CareStart™ (AccessBio, USA) was performed according to the manufacture's instruction. Test showing a pink color were classified as normal, tests showing no or very faint color were classified as deficient. The G6PD spectrophotometric assay (Trinity Biotech, Ireland) was performed according to the supplier instruction. An UV spectrophotometer (UV mini-1240, SHIMADZU, Japan) with electronically controlled temperature compartment was used to detect the absorbance at 340nm. The overall prevalence of G6PD deficiency in this study was 6.8% in the whole population and 11.1% in males (see table 1). Deficiency was found in 6.6% (50 persons) of all Bamar subjects (10.1% in males) and 9.2% (11 persons) of all Karen subjects (21% in males). The haemoglobin types of study population were shown in table 2. FST (Fluorescent spot test) and CareStart™ test showed comparable performances with sensitivity over 95% and specificity over 90%. The CareStart™ test has the potential to be used in the field for a wider use of primaquine in malaria elimination in Myanmar.

Table 1. G6PD deficiency prevalence in study population

	Mean Age ±SD (years)	G6PD Deficiency	Anaemia	Reticulocytes Mean±SD
Women (n=476)	39.1±12.4	10 (2.1%)	77 (16.2%)	0.57±0.70
Men (n=524)	37.6±13.6	58 (11.1%)	13 (2.5%)	0.58±0.91

Table 2. Different haemoglobin types in study population

Haemoglobin Type	Number	RBC Mean±SD	HGB Mean±SD	MCV Mean±SD	Anaemia	Reticulocytes Mean±SD (p<0.001)
AA (Normal)	821 (82.1%)	5.36 ±0.67	13.7 ±1.8	80.3 ±6.6	64 (7.8%)	0.59 ±0.82
AE (Heterozygous)	169 (16.9%)	5.7 ±0.80	13.6 ±1.9	74.1 ±7.01	21 (12.4%)	0.5 ±0.83
EE (Homozygous)	7 (0.7%)	6.21 ±0.22	11.1 ±2.7	56.0 ±3.6	5 (71.4%)	0.46 ±0.22

*Three subjects with HbAD and unknown Hb type were not included.

2. NON-COMMUNICABLE DISEASES

2.1 SNAKE BITE

2.1.1 Characterization of Hyaluronidase isolated from Russell's Viper (*Daboia siamensis*) Venom of Myanmar

The spreading factor, hyaluronidase enzyme is one of the components of snake venom which contributes to local damage at bite site by affecting the extracellular matrix and potentiates the toxicity of venom. Hyaluronidase cleaves the major component of extracellular matrix (ECM) of vertebrates' hyaluronic acid (HA), which connects protein filaments, collagen fibers and the connective tissue cells. It increases tissue permeability and enhances the rate of venom absorption. This study was conducted with an aim to assess the characterization of hyaluronidase enzyme isolated from Russell's Viper (*Daboia siamensis*) Venom (RVV) of Myanmar. Firstly, hyaluronidase enzyme was isolated from crude RVV by using HiTrap SP FF strong cation exchange column coupled with AKTA Start 1.0 FPLC system. The chromatographic profile of RVV showed 8 different major peaks. Hyaluronidase activity was then determined by Pukrittayakamee method. Hyaluronidase enzyme activities were detected only at peak 6 and 7. The enzyme was partially purified to 1.88 fold with a yield of 3.11%. The turbidity reduction units of crude RVV, peak 6 and 7 were found to be 47.43, 25.17 and 25.48 respectively. By using ELISA method, it was noted that the specific monovalent anti snake venom (ASV) (1:400 dilution) could not completely inhibit the hyaluronidase enzyme fraction (11.37%). It was also confirmed by Western blot analysis in which the ASV (1:3200 dilution) could not totally recognize the hyaluronidase containing fraction. Therefore, it could be concluded that anti-hyaluronidase activity was not detected in locally produced ASV. It may be due to lack of heat-labile hyaluronidase enzyme in crude venom used in immunization of horse or less immunogenic property of enzyme itself. It is suggested that improvement of methodology for production of potent anti-snake venom needs to be considered for achieving a more potent ASV, leading to reduction of morbidity and mortality resulted from snake bite in our country.

3. ENVIRONMENTAL HEALTH

3.1 Determination of T-2 Mycotoxin in cereal-based junk foods sold from some food stalls in Yangon Region

(Refer to annual report of Biological Toxicology Research Division)

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Thet Thet Mar	1 st year MMedSc (Biochemistry), UM(1), UM(2) and DSMA. 1 st year MMedSc (Pharmacology), UM(1),UM(2), UMM, UM (Magway) & DSMA. 1 st year MPharm, University of Pharmacy 1 st year MMedTech, University of Medical Technology Research Methodology Workshop (2015)	Teaching Facilitator
2.	Daw Lwin Zar Maw	1 st year MMedSc (Biochemistry), UM(1), UM(2) and DSMA.	Demonstration of SDS-PAGE

BIOCHEMISTRY RESEARCH DIVISION (POL)

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Research Officers	...	Dr. Aye Min Maw MBBS, MMedSc (Pharmacology)(UMM)
	...	Dr. Thet Oo Wai MBBS(UMM)
Laboratory Incharge	...	Daw Khaing Khaing Mar BSc(Chemistry)(MU)
Research Assistant (2)	...	Daw Saw Ohnmar Khin BSc(Physics)(MU)
	...	Daw Kyawt Kyawt Khaing BSc(Chemistry)(MU)
	...	Daw Thandar Myint Thaw BPharm(UOP, Mandalay)
	...	Daw Khin Lay Sein BPharm(UOP, Mandalay)
	...	Daw Kyi San BSc (Physics)(Meikhtila University)
Research Assistant (3)	...	Daw Aye Thi
	...	Daw Nu Yi Thin BSc (Biochemistry)(Magway University)
Research Assistant (4)	...	U Aung Thu Ra BSc (Physics)(MU)
	...	Daw Lei Lei Win BSc(Zoology) (Monywa University)

Biochemistry Research Division has been actively engaged in conducting a number of research projects in areas of non-communicable diseases and traditional medicine. Other services of supervision of Ph.D, M.Med.Sc, M.Res and M.Sc candidates were also provided to University of Medicine, Mandalay and Mandalay University.

RESEARCH PROJECTS

1. NON-COMMUNICABLE DISEASES

1.1 CANCER

1.1.1 Biochemical and hematological profiles of breast cancer patients attended to Out-Patient Department of cancer unit, Mandalay General Hospital

The biochemical alteration of the cancer cell in an extreme situation could lead to a complete deletion of a critical macromolecule, resulting in something akin to a localized and acquired error of metabolism. This hospital and laboratory-based, cross-sectional comparative study was conducted to assess the biochemical and hematological profiles including glucose, hepatic enzymes, renal biomarkers, uric acid, zinc and blood for complete picture of 36 newly-diagnosed cases of female breast cancer patients from Out-Patient Department of Cancer Unit in Mandalay General Hospital. The parameters were compared before first cycle and after third cycle of chemotherapy. Then, these parameters measured before chemotherapy were compared with 36 of apparently healthy control subjects. It was found that GPT, creatinine, neutrophil and monocyte levels of patients before first cycle of chemotherapy were significantly higher than those of control subjects whereas urea, uric acid, Hb% and lymphocyte levels of patients before chemotherapy were significantly lower than those of control subjects. Uric acid, zinc, neutrophil and eosinophil levels of patients before first cycle of chemotherapy were significantly higher than after third cycle of chemotherapy whereas lymphocyte and monocyte levels of patients before chemotherapy were significantly lower than after third cycle of chemotherapy. According to these results, some biochemical and hematological parameters were different between control and before chemotherapy and

between before and after chemotherapy. However, they all were within normal values. This may be due to rehydration, correction for reduction of hemoglobin and white cell counts and rebuilt management during chemotherapy. In this study, study limitations were the withdrawal of patients before third cycle of chemotherapy and limited study period leading to obtaining insufficient sample size. Therefore, biochemical and hematological parameters should be monitored during management of breast cancer for effective supportive treatments.

1.1.2 Assessment of serum selenium levels in breast cancer patients

Selenoproteins have roles that support immune function and, through specific cellular pathways, may play a preventive role in both the initiation and promotion of specific cancers. Selenium exerts its chemoprevention effect in different ways, such as a protective effect against oxidative damage by decreasing the amount of free radicals and increasing the synthesis of glutathione peroxidase. In Myanmar, breast cancer is annually increasing during these years. Therefore, the present work was taken up to assess serum selenium levels in breast cancer patients before first cycle of chemotherapy and after third cycle of chemotherapy. This hospital and laboratory-based, cross-sectional comparative study was conducted at Cancer Unit in Mandalay General Hospital. Study population was 37 new cases of female breast cancer patients above 20 years of age who did not suffer from any major illness in the past. Serum selenium was measured by using Atomic Absorption Spectrophotometer. Our finding showed that mean serum selenium level in breast cancer patients was significantly lower than that of controls and significantly higher than that measured after third cycle of chemotherapy. From the obtained results, it could be concluded that, the deficiency of selenium may lead to increase in risk of cancer incidence and effect of chemotherapy may increase reactive oxygen species with decreasing antioxidant activity.

1.2 METABOLIC SYNDROME

1.2.1 Metabolic risk factors and associated morbidities among rural community

The metabolic syndrome is a clustering of inter-related risk factors that identify individuals at increased risk for type 2 diabetes mellitus and cardiovascular disease. This laboratory-based, cross-sectional descriptive study was done to identify metabolic risk factors and associated morbidities among rural community. Total 263 participants from Pin Tee Village, Pyin Oo Lwin Township were assessed in this study. In order to make a diagnosis of the metabolic syndrome according to ATP III clinical identification, a patient must present with three or more of the following five risk factors: (1) central obesity (males: Waist circumference > 102 cm ; females: > 88 cm) (2) raised plasma triglycerides (≥ 150 mg/dL) (3) low HDL cholesterol (<40 mg/dL men; <50 mg/dL women) (4) raised arterial pressure $\geq 130/85$ mmHg (5) fasting blood sugar ≥ 110 mg/dL. According to BMI, 6.7% of participants were ≤ 18.4 , 71.1% were within 18.5-24.9 and 22.2% were >24.9 . Measuring the blood pressure, 82.2% were normal and others (17.8%) were raised in blood pressure. It increased with increasing age. ECG results were normal among 220 respondents (82%) and the others (18%) were found to have abnormal changes. Assessing the biochemical profile, fasting blood sugar levels of 9.5% participants were higher than normal range. Only 8% of the participants had high cholesterol level, 31.9% had high triglyceride level, 4.2% had high LDL level, and 27.8% had low HDL level. Regarding the liver function test, most of the participants were normal in total bilirubin level (91.3%) and ALP level (93.9%) otherwise ALT and AST levels were abnormal in most participants, 87.1% and 82.2% respectively. Urea and serum creatinine levels were also measured and the results were normal in 85.2%

and 77.6% respectively. The participants of 72.2% were normal in serum uric acid level. According to this study, rural community has low metabolic risk factors.

2. TRADITIONAL MEDICINE

2.1 HEAVY METALS

2.1.1 Study of some heavy metals contamination in two *Tinospora* (ဆင်တုံးမနွယ်) species

Medicinal plants are the most important source of life saving drugs for the majority of the world population. World Health Organization (WHO) estimates that more than 80% of people in developing countries depend on traditional medicine for their primary health needs. Heavy metals are metallic elements with high atomic number and poisonous to living organisms. Plants may absorb heavy metals from soil, water or air. The purpose of present study was to determine the heavy metals contamination in *Tinospora cordifolia* (ဆင်တုံးမနွယ်ပြောင်ချော) and *Tinospora crispa* (ဆင်တုံးမနွယ်ဆူးပေါက်) which are reputed for treatment of anti-diabetic, anti-inflammatory, antiarthritic, antispasmodic and antiallergenic. The atomic absorption spectrophotometer (AAS) was used for determination of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in two *Tinospora* species and their surrounding soils from Mandalay, Pyin Oo Lwin and Shwe Bo. These two *Tinospora* species and all soils contained metals, which were within permissible limits except Fe content. The plants contained higher amount of Fe ranged between 22.22 to 57.32 ppm. However, this study was comparable to the study reported by Khin Phyu Phyu *et al.* (Jan, 2014), 'Fe' ranged between 76.78-356.05 ppm, and Jabeen *et al.* (2010), the range of 'Fe' in selective medicinal herbs of Egypt in the study carried out was between 261 to 1239 ppm, respectively. These findings obtained from the present study can provide scientific data which will be helpful for herbal medicine users, local practitioners and pharmaceutical industries using these two *Tinospora* species for different types of ailments. In conclusion, monitoring such medicinal plants for heavy metals is applicable for references and supreme importance in protecting the public from adverse and hazardous effects of heavy metals.

2.2 DIURETIC EFFECT

2.2.1 The diuretic effects of the whole plant and fruits of *Tribulus terrestris* Linn. (ဆူးငေ့) in Wistar albino rats

The medicinal plants are major component in traditional medicine as well as valuable potential resource for new drugs. *Tribulus terrestris* Linn. (ဆူးငေ့) is one of the medicinal plants used for diuretic, hypotensive, antiurolithiatic and spasmolytic activities in traditional medicine of many countries including Myanmar. This study aims to prove scientifically the diuretic effect of *Tribulus terrestris* Linn. by comparing the effects of the watery extracts of the whole plant and fruits. Qualitative phytochemical analysis revealed that both whole plant and fruits extracts contained carbohydrate, reducing sugar, glycoside, saponin and amino acid but alkaloid and tannin were present only in the fruit. Acute oral toxicity test was done in ICR mice by using OECD 425 guideline (2008) and there were no acute toxic and lethal effects with dose of 5000 mg/kg in both samples ($LD_{50} > 5000$ mg/kg). The study design is comparative randomized controlled experimental animal study. Eight groups of 6 Wistar albino rats in each were used for this study. Watery extract of 3 different doses (300 mg/kg, 500 mg/kg and 700 mg/kg) for each whole plant and fruits were used to test diuretic effect. Control group was given 0.9% sodium chloride solution and furosemide was used as the standard drug. After fasting, the animals were given watery extract orally and put into

metabolic cages. Then urine was collected for 5 hours. Urinary sodium and potassium concentrations were measured by atomic absorption spectrophotometer (AAS). When analyzing the results, as compared with control, there was no significant diuretic effect in whole plant extract but only fruit extract showed significant diuretic effect.

2.3 NUTRITION

2.3.1 Determination of macronutrients, micronutrients and antioxidant properties of *Zingiber officinale* (ginger) and *Foeniculum vulgare* (fennel)

Medicinal plants are commonly used in a large number of medicines for treatment of different types of diseases. World health organization (WHO) estimated that more than 80 % of the world's population consumes indigenous medicinal plants in direct and indirect ways to treat their diseases. Micronutrient malnutrition is a major global health concern because its deficiency in the body is linked with ill health and diseases. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The purpose of the current study was to investigate the macronutrients, micronutrients and antioxidant activities of *Zingiber officinale* (ginger) and *Foeniculum vulgare* (fennel). Atomic absorption spectrophotometer and UV Visible spectrophotometer were used for determination of macro & micronutrients and antioxidant activities of ginger and fennel. The results of macronutrients were Ca (291 - 2007 ppm), Mg (40.61 - 42.27 ppm), Na (83.59 - 87.96 ppm) and K (727.92 - 738.36 ppm) respectively. The micronutrients including Cu, Fe, Mn and Zn were ranged within 2.38-10.18 ppm, 78.75-209.57 ppm, 44-168 ppm and 11- 22 ppm respectively. Considering the antioxidant activity, this study revealed that both ginger and fennel have free radical scavenging activity. These results obtained from current study indicated that, ginger and fennel have potential to provide nutrients to human beings, preventive properties against oxidative damage.

SERVICES PROVIDED

LABORATORY

Sr. No.	Laboratory tests	Tested samples
1.	Cadmium level in water by atomic absorption spectrophotometer (AAS)	51 samples
2.	Lead level in water by AAS	48 samples
3.	Serum zinc level by AAS	16 samples
4.	Serum lipid profile by biochemical analyzer	55 samples
5.	Fasting blood sugar	365 samples
6.	Liver function tests (Total bilirubin, ALT, AST, ALP)	365 samples
7.	Urea	365 samples