BIOCHEMISTRY RESEARCH DIVISION

Deputy Director & Head	...	Dr. Thet Thet Mar BSc(Hons), MSc, PhD(Chemistry)(YU)
Research Scientist	...	Dr. Khin Than Yee MBBS, MMedSc (Biochemistry)(UM1)
Research Officers	...	Dr. Thuzar Hla Shwe MBBS (UM2)
	...	Dr. Hsu Mon Aung MBBS (UM1)
	…	Daw Lwin Zar Maw BSc (Chemistry)(UDE), DFT(YU)
Research Assistant (4)	...	Daw Nwe Ni Aung BSc (Biotechnology)(DU)
Laboratory Attendant	...	Daw Yi Yi Sein

The Biochemistry Research Division is actively involved in research activities of Non-Communicable diseases such as Snake bite and Diabetes.

RESEARCH PROJECTS

1. NON-COMMUNICABLE DISEASES

1.1.DIABETES MELLITUS

1.1.1 Serum asymmetric dimethylarginine (ADMA) level in diabetics and prediabetics

A cross-sectional comparative study was carried out on known diabetic patients attending the diabetic clinic of Yangon General Hospital and apparently non-diabetic individuals. The study was conducted from July to October 2016 and a total of 92 participants aged 30 to 65 years were recruited. The objectives of the study were to determine and compare serum Asymmetric Dimethylarginine (ADMA) levels among diabetics, prediabetics and normoglycemic participants. Fasting blood sugar (FBS) and 2-hour-post-glucose blood sugar (2hPG), if necessary, were determined using glucometer to categorize the participants. Then, blood samples were collected for measurement of serum asymmetric dimethylarginine. Out of 92 participants, 30 diabetics, 34 prediabetics and 28 normoglycemic participants were involved in this study. Sixty six participants (71.7%) were female. Mean age of study population was 51.79±10 years while that of diabetic, prediabetics and normoglycemic groups were 54.73 ± 8.5 years, 52.85 ± 9.1 years and 47.36 ± 11.6 years, respectively. Only 17.4% (16/92) participants had a BMI within normal range (17.5-22.9) while over half of participants (47/92) were within overweight range of BMI 23-27.9 and 31.5% (29/92) of participants were obese (BMI = ≥ 28).

	Diabetics (n=30)		Prediabetics (n=34)		Normoglycemics (n=28)		p value
	Mean	SE of mean	Mean	SE of mean	Mean	SE of mean	
FBS (mg/dL)	178.50	11.89	105.21	1.20	91.86	0.96	0.000
2hPG (mg/dL)	-	-	149.56	3.89	110.89	3.17	0.000
ADMA (µmol/L)	0.67	0.06	0.85	0.07	0.61	0.04	0.014

There were significant differences among mean values of serum asymmetric dimethylarginine (ADMA) of 3 groups; Diabetics, Prediabetics and Normoglycemics (p = 0.014). Prediabetic participants had significantly higher ADMA level than normoglycemic

one (p = 0.005) although difference of ADMA level between Diabetics and Normoglycemics groups was insignificant (p = 0.44). Moreover, there was a significant correlation between ADMA level and BMI in total population (p = 0.020) despite there was no significant difference between mean BMI of 3 groups. This study revealed that the endothelial dysfunction marker, ADMA level was significantly increased in prediabetic individuals and those with high BMI. Serum ADMA level can be used as one of the early cardiovascular risk assessment factors in prediabetic individuals. This study highlighted the increased cardiovascular risks in apparently healthy Prediabetic individuals, thereby, raised the awareness to carry out early risk determination and reduction measures in the community.

1.2. SNAKE BITE

1.2.1 Detection of Russell's Viper (*Daboia siamensis*) Venom concentration in experimentally envenomated animals' blood by ELISA method

The incidence of snake bite in Myanmar is estimated for more than 10,000 per year with an average case fatality rate of 7.4%. Among 312 Russell's Viper bite cases of six township hospitals (Danubyu, Yegyi, Nyaunglaybin, Myinmu, Kyaukse and Taungdwingyi), 28% (87/312) of victims had no envenomation (blank bite), 29%(91/312) was local, and 43%(134/312) was systemic envenomation leading to complications. The aim of study is to detect the Russell's Viper (*Daboia siamensis*) Venom (RVV) concentration in experimentally envenomated animals' blood by ELISA and western blot method. Fifty adult mice ddy strain (*Mus muculus*) (20-32g) were intraperitoneally (*i.p*) injected with $3LD_{50}$ (900µg/kg) dose of *Daboia siamensis* venom. After injection of venom, the mice were randomly divided into 5 groups (each group containing 10mice). Blood samples were taken from the heart of each group into plain tubes at various time intervals: 1hr, 2hr, 3hr, 4hr and 5hr respectively. Placed the blood tubes at 4ºC for 1 hr and then centrifuged for 10 mins at 2500rpm. The amounts of venom were measured by ELISA method, using a volume of 100µl of samples and a series of venom standards (ranging from 0.975ng to 500ng/100µl diluted in normal serum). Sensitivity of the detection of *Daboia siamensis* venom by ELISA was determined by using Cobra (*Naja kaouthia*) venom. The same venom levels of Cobra venom (ranging from 0.975ng to 500ng/100µl diluted in normal serum) were added into anti-*Daboia siamensis* venom coated wells. The anti-*Daboia siamensis* venom coated ELISA plate give the signals only different levels of *Daboia siamensis* in normal serum and does not give any signal of different levels of *Naja kaouthia venom* in normal serum. The detected mean levels of *Daboia siamensis* venom were 58.1, 171.2, 42.9, 127.33 and 22.1ng/mL at 1, 2, 3, 4 and 5hrs respectively. The different levels of RVV and *Naja kaouthia* in normal serum were measured by direct blot onto PVDF membrane and detected with horse anti-*Daboia siamensis* venom. The RVV in blood can detect qualitatively and horse anti-*Daboia siamensis* venom does not cross react with *Naja kaouthia* by direct blot method. In conclusion, the RVV concentration in blood samples can be quantatively and qualitatively detected in envenomated victims' blood by ELISA method and direct blot onto PVDF membrane method. The detection and quantitation of RVVs are helpful for both diagnosis and prognosis of snake envenomation in Myanmar.

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Thet Thet Mar	1st year MMedSc (Biochemistry), DSMA. 1st year MMedSc (Pharmacology), UM1, UM2, UM (Magway) & DSMA. 1st year MPharm, University of Pharmacy	Teaching, Demonstration of Column Chromatography, ELISA and Western Blot Lecturer "Blood Sample Collection"
2.	Daw Lwin Zar Maw	Training of Trainers on "Myanmar Micronutrient and Food Consumption Survey" 1st year MMedSc (Biochemistry), DSMA.	Demonstration of SDS-PAGE

BIOCHEMISTRY RESEARCH DIVISION (POL)

Deputy Director & Head	…	Dr. Kyae Mhon Htwe MBBS, MMedSc (Biochemistry) (UMM)
Research Scientist	…	Daw Lei Lei Win BSc, MSc (Chemistry) (MU)
Research Officer	…	Dr. Aye Min Maw MBBS, MMedSc (Pharmacology) (UMM)
	…	Dr. Thet Oo Wai MBBS (UMM)
	…	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 Thura 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 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), University of Traditional Medicine (Mandalay) and Mandalay University.

RESEARCH PROJECTS

1. TRADITIONAL MEDICINE

1.1 ANTIBACTERIAL EFFECT

1.1.1 Determination of bioactive components from *Foeniculum vulgare* (fennel) (စမုံစပါး) seeds and antibacterial activities of *Zingiber officinale* (ချင်း)

Medicinal plants are potential sources of natural compounds with biological activities and therefore attract the attention of researchers worldwide. The purpose of current study was to determine bioactive components from *Foeniculum vulgare* (fennel) (စမုံစပါး) and antimicrobial activities of *Zingiber officinale*, ginger(ချင်း).Bioactive components present in *Foeniculum vulgare* (fennel)(စမုံစပါး) seeds was determined by using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis was extremely useful in identification of compounds and based on peak area, retention time, molecular formula, molecular weight, MS Fragment- ions and pharmacological actions. Aqueous extracts of fennel seed contained 4-methoxy- Benzaldehyde, N- (1- clohexylethyl) Propanamide, Nonanoic acid,9-oxo-methyl ester, 2-(methylaminomethyl)-trans-Cyclohexanol, Hexadecanoic acid methyl ester, 9,12-Octadecenoic acid (Z,Z) methyl ester, 9-Octadecenoic acid (Z), methyl ester, Docosanoic acid, methyl ester, Tetracosanoic acid, methyl ester and one unknown compound.1,3,3-trimethyl Bicyclo [2.2.1] heptan-2-one, 4-methoxy- Benzaldehyde, 1-methoxy-4-(1-propenyl)- Benzene (Anethole), alpha- (1-ethanylpentyl)-alpha-methyl Benzenemethanol, Nonanoic acid 9-oxo - methyl ester, Hexadecanoic acid methyl ester, 9,12-Octadecenoic acid (Z,Z) methyl ester, 9-Octadecenoic acid (Z) -, methyl ester, Docosanoic acid and two unknown compounds were identified in ethanolic extract of fennel seeds.4-methoxy-Benzaldehyde, Nonanoic acid, 9-oxo-methyl ester, Hexadecanoic acid, methyl ester, 9,12-

Octadecenoic acid (Z,Z), methyl ester, Docosanoic acid, methyl ester contained both aqueous and ethanolic extracts of fennel seed. Antibacterial activity of ginger was determined by agar disc diffusion method (WHO, 2003). Broth dilution method was used for determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Both aqueous and ethanolic extracts of ginger showed zone of inhibition 12 mm – 15 mm against *Pseudomonas aeruginosa,* 7mm - 9 mm against *Staphylococcus aureus* and 12 mm – 15 mm against *Escherichia coli*. Although MIC and MBC of aqueous extracts did not show the activity at the concentration up to 10 mg/mL, MIC of ethanolic extract was observed in the range from 1 mg/mL to 8 mg/mL and MBC from 2 mg/mL to 9 mg/ml. Therefore, antibacterial activity of ginger was more potent in ethanolic extract. These results can provide scientific information for herbal medicine users, local practitioners and pharmaceutical industries using fennel and ginger for different types of ailments.

1.2 DIURETIC EFFECT

1.2.1 Safety and diuretic effect of *Arundo donax* Linn. (အလိုကျွဲ)

From ancient time, plants are rich source of effective and safe medicines. Herbal medicines are finished, labeled medicinal products that contain as active ingredients, aerial or underground part of plants or other plant materials, or combination, whether in the crude state or as plant preparations. This study aimed to evaluate scientifically the safety and the diuretic effect of *Arundo donax* in Wistar albino rats. Its rhizome is used as an ingredient in traditional medicine formulation (21) (TMF-21) (ဆီးဆေးဖြူ), Urocrush. The aqueous extract of rhizome of *Arundo donax* Linn. contained alkaloid, glycosides and reducing sugar as phytochemical constituents. The acute oral toxicity of that extract was done according to OECD 425 guideline (2008) in ICR mice and it revealed that LD_{50} was > 5000 mg/kg. For the diuretic activity, 30 Wistar albino rats were used and they were divided into 5 groups of 6 animals in each. Those 5 groups were administered 0.9% NaCl as control, furosemide as standard and 3 doses of aqueous extracts (125 mg/kg, 250 mg/kg and 500 mg/kg) as tests respectively. After administration, each animal was placed individually in metabolic cage for 5 hours to determine urine output and urinary sodium and potassium concentrations were measured by atomic absorption spectrophotometer. Among 3 test doses, 250 mg/kg dose significantly increased urine output and excretion of urinary sodium and potassium ($p< 0.05$) compared with control. This finding can give scientific information for traditional medicinal practitioners and users.

SERVICES PROVIDED

LABORATORY

Sr. No.	Laboratory tests	Tested samples
1.	Cadmium level in water by atomic absorption spectrophotometer (AAS)	3samples
2.	Lead level in water by AAS	10 samples
3.	Serum zinc level by AAS	154samples
4.	Serum selenium level by AAS	53 samples