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CONTENTS

Pharmacological and chemical studies on <i>Orthosiphon aristatus</i> (Bl.) Miq.....	1
<i>Aye Than, Win Myint, Tin Myint, Mu Mu Sein Myint, Than Than Htay & Khin May Ni</i>	
Analgesic effect of Chin saw kha thee (<i>Cydonia cathayensis</i> Hemsl.) on experimentally induced pain in human subjects.....	7
<i>May Aye Than, Mu Mu Sein Myint, Aye Than, Kyi Kyi Myint, San San Myint, Thazin Myint & Tin Nu Swe</i>	
Existing practices of general practitioners on diagnosis and treatment of tuberculosis in Yangon.....	12
<i>Saw Saw, Mon Mon, Soe Naing, Kyi Kyi Mar, San San Aye, Win Win Mar & Zin Mar Aye</i>	
Purgative effect of Pway-mezali (<i>Cassia alata</i> Linn.) leaves on healthy subjects.....	17
<i>May Aye Than, Mu Mu Sein Myint, Aye Than, Myint Thuzar Thant, Thandar Myint, & Tin Nu Swe</i>	
Experiences and knowledge of malaria among hospitalised patients of Myanmar.....	22
<i>Cho Cho Oo, Htein Lin & Alan Pearson</i>	
Safety and efficacy of coloured liquid Russell's viper (<i>Daboia russelli siamensis</i>) antivenom manufactured by Myanmar Pharmaceutical Factory.....	27
<i>Tun Pe, Aye Aye Myint, Kyi May Htwe & Khin Pyone Kyi</i>	
Stability of potency of Russell's viper (<i>Daboia russelii siamensis</i>) antivenom on storage.....	31
<i>Tun Pe, Aye Aye Myint & Kyi May Htwe</i>	
Bacteriological aspects of milk and milk products in Yangon during 2000.....	35
<i>Mar Mar Nyein, Thet Thet Khine & Kyi Kyi Thin</i>	
Venom ophthalmia following spitting of venom into the eyes by spitting cobra (<i>Naja mandalayensis</i>) in Myanmar.....	42
<i>Tun Pe, That Htut, Aye Aye Myint & Myo Tint Tun</i>	

45 days mechanical ventilation in a Chinese krait (<i>Bungarus multicinctus</i>) bite	45
<i>Tin Myint & Tun Pe</i>	
Short Report : Social aspects of leprosy patients in Hmawbi Township.....	48
<i>Kyaw Myint, Le Le Win, Khin Myint Wai & Mya Pwint Aye</i>	
Short Report : Batch to batch variations in Russell's viper (<i>Dabola russelii siamensis</i>) venom.....	50
<i>Aye Aye Myint, Tun Pe, Kyi May Htwe, Khin Aung Cho & Theingie</i>	

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Pharmacological and chemical studies on *Orthosiphon aristatus* (Bl.) Miq.

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The plant *Orthosiphon aristatus* (Bl.) [See-cho-pin or Tha-gyar-ma-gike (ဆီးချိုပင်, သကြားမကိုင်း) in Myanmar name] scientifically evaluated for its traditionally reputed activity of hypoglycemic property. When aqueous extract of the leaf was tested on rabbit model, blood sugar lowering effect was observed on both adrenaline-induced and glucose-loaded models. Reduction in blood glucose concentration was significant and was maximum at 1hr and 2hr. Acute toxicity test and physico-chemical studies of the leaf extracts of the plant were also conducted. The acute toxicity studies carried out on mice has not revealed an adverse or side effects of this extract at the dosages tested. Detailed results on the evaluated hypoglycemic activity, acute toxicity study and physico-chemical tests were discussed and reported.

INTRODUCTION

The discovery of plants with promising pharmacological activities has being widely explored in medicinal plant research leading to the development of important natural drugs or pharmacological tools. On the other hand, chemical screening of medicinal plants used in traditional pharmacopoeia of some countries was being undertaken without experimental evaluation of specific pharmacological activities [1]. There were also a great number of experiments working on the specific pharmacological activity with the responsible active chemical constituents in the area of medical plant research. In Myanmar, selected traditionally reputed medicinal plants have been evaluated experimentally and a few number of promising plants were clinically and chemically investigated.

Screening of the medicinal plants for hypoglycemic activity is one of the

important test in plant drug research. A series of experiments had already been conducted on the blood sugar lowering effect of *Momordica charantia* L. fruit (bitter gourd) contain active principle polypeptide-p from plant source [2,3,4]. An interesting plant with the local name of See-cho-pin is worth evaluating for its hypoglycemic property due to the long traditional usage in hyperglycemia as a herbal medicine. The present investigation was, therefore, undertaken to evaluate the effects of aqueous extract of the leaves in diabetes induced rabbit models for the hypoglycemic property and to characterize the physico-chemical properties of the plant.

MATERIALS AND METHODS

Preparation of leaf extracts

Before preparation of the extracts, the plants were collected within the Yangon Division

and botanically identified and authenticated by a taxonomist from the Department of Botany, Yangon University. It was confirmed as *Orthosiphon aristatus* (Bl.) Miq.

Crude aqueous extract was prepared by refluxing the leaf powder with distilled water on a boiling water bath for about 6 hours. It was then cooled and filtered. The filtrate was evaporated to dryness by rotary evaporator and was totally dried by leaving the extract under vacuum overnight. The yield was shown to be 1.9 g % (w/w). The extract solution was prepared by dissolving the required extract in 1% methylcellulose solution for having specific concentration before administering to an animal for studying hypoglycemic activity.

Physicochemical and phytochemical analysis

Physicochemical parameters such as moisture content (by moisture tester, Ultrax); total nitrogen content (by micro Kjeldhal method); successive extractive values; total alcohol and water soluble matters, essential oil content (by volatile oil apparatus); ash values and mineral concentrations (by atomic absorption spectrophotometer, PYE UNICAM) were determined by standard methods [5,6,7,8]. Qualitative tests for the detection of organic compound were conducted by the method as described in the reference [1]. Previously reported compounds of *O. aristatus* such as sinensetin (flavonoid) [9] and β -sitosterol (steroid) were also detected by thin layer chromatography (TLC) which further contributed in for the scientific identification of the plant.

Effect on adrenaline-induced hypoglycemia in rabbit

Six rabbits were fasted overnight with free access to water. The blood samples were collected from marginal ear vein to determine baseline blood sugar levels. They were made hyperglycemic by injecting them subcutaneously with 10 mg/kg of adrenaline

tartrate injection [10]. To act as control, the test animals were administered with 1% of methylcellulose at a dosage of 10 ml/kg respectively. Serial blood samples were collected at 1hr, 2hr and 4hr, and the blood sugar levels were estimated by orthotoluidine method [11]. The rabbits not responding to adrenaline injection were excluded while the responding rabbits were fasted again after a week and administered with 2 g/kg of the aqueous extract orally with the help of gastric tubes.

Effect on glucose-loaded hyperglycemia in rabbit model

Six rabbits of both sexes were weighed and their base line blood samples were collected. Then they were administered with 3g/kg glucose solution dissolved in 1% acacia at a concentration of 3g/10 ml. The oral glucose tolerance test (OGTT) was carried out [10, 12]. The blood samples were collected serially at 1hr, 2hr and 4hr respectively to determine the blood sugar levels using O-toluidine method as mentioned previously. The rabbits not responding to glucose administration were excluded while the responding rabbits whose blood sugar levels increased after administration of glucose were chosen for the test group. After a week interval, test animals were administered 3g/kg glucose dissolved in 1% acacia together with the watery extract 2g/kg dissolved in 1% acacia. After a time course of peak interaction, blood samples were again taken from each animal and blood sugar levels were again determined as mentioned previously.

Acute toxicity study

Ten albino mice in each group were fasted overnight before administration of the plant extract. Increasing doses of the extract in 1% methylcellulose, 2, 4 and 8 g/kg were orally administered. The mice were housed separately in individual aluminium cage with free access to food and water and observed clinically for 1 week. LD50 was

determined from the number of animals surviving at the end of 1 week period [13].

RESULTS AND DISCUSSION

Morphology, taxonomy and anatomy of the plant were observed to agree with *Orthosiphon aristatus* and were documented at the Department of Botany, Yangon University. The morphology of the plant was shown in Figure 1.

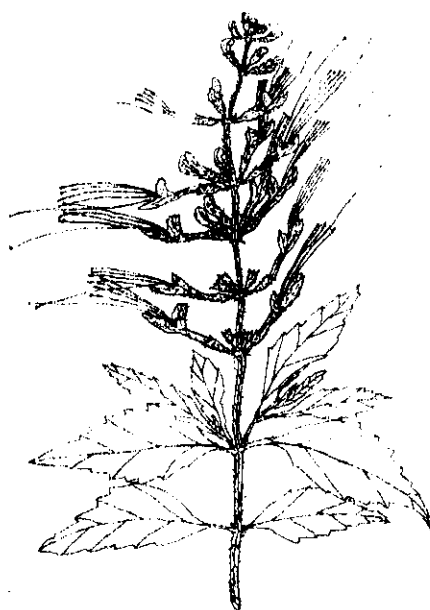


Fig.1. Habitat sketch of *Orthosiphon aristatus* (Bl.) Miq.

Physico-chemical data of the dried leaf powder was tabulated in Table 1 and qualitative identification tests on various extracts were shown in Table 2.

Detection of sinensetin and β -sitosterol from the leaves determined by TLC study were described in Figure 2, Figure 3, Table 3 and Table 4 respectively. The botanical and chemical studies authenticated and confirmed the scientific name of See-chopin or Tha-gyar-ma-gike as *Orthosiphon aristatus* Bl. (Miq.).

Table 1. Physico-chemical data of dried leaf powder

Test parameter	Results
Moisture content	10.8%(Dec), 9.2%(April)
Total nitrogen content	3.57%
Total water soluble matters	7.56%
Total alcohol soluble matters	16.32%
Successive extractive values:	
• Pet-ether soluble extracts	3.00%
• Chloroform soluble extracts	5.28%
• Ethanol soluble extracts	2.10%
• Total ash	13.35%
• Acid insoluble ash	0.60%
• Water soluble ash	7.45%
• Essential oil content	0.02% (v/w)
Mineral contents	
• Sodium (Na)	0.04%
• Potassium (K)	2.77%
• Calcium (Ca)	0.18%
• Copper (Cu)	0.001%
• Iron (Fe)	0.201%
• Zinc (Zn)	0.009%

Table 2. Results of qualitative test on *Orthosiphon aristatus*

Organic constituents	Successive extracts			Total extract	
	P-ether ext	CHCl ₃ ext	EtOH ext	EtOH ext	H ₂ O ext
Alkaloids	+	+	+	-	-
Flavonoids	+	+	+	+	+
Steroids	+	-	-	-	-
Glycosides	+	-	+	+	+
Amino acids	-	-	-	+	-
Protein	-	-	-	+	+
Carbohydrate	-	-	+	+	+
Saponins	-	-	-	-	-
Tannins	-	-	+	+	+
Phenolic compounds	+	+	+	+	+
Resin	-	-	-	-	-
Starch	-	-	-	-	-

Table 3. TLC data of reference sinensetin and *O. aristatus* extracts

Sample	Colour	R _f value
Sinensetin, Ref:	bright white blue	0.60
Petroleum ether extract	bright white blue	0.53
Alcohol extract	bright white blue	0.54
Chloroform extract	bright white blue	0.56
Dichloromethane extract	bright white blue	0.57

Table 4. R_f value of standard β -sitosterol and analysed spot of *O. aristatus* leaves

Sample	R_f		Colour
	Solvent I	Solvent II	
Standard β -sitosterol	0.86	0.25	Brown
Analysed spot of <i>O. aristatus</i>	0.86	0.25	Brown

Solvent I = 1 Hexane: 1 Ethyl acetate

Solvent II = 1 Benzene: 1 Chloroform

Sprayed reagent = Libermann - Burchard

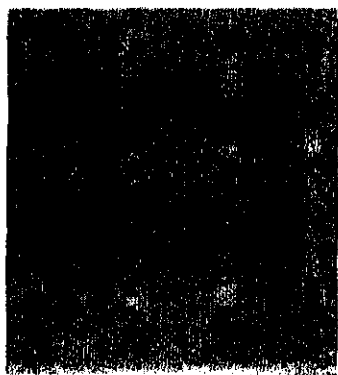


Fig. 2 Thin layer chromatogram of sinensetin in *Orthosiphon aristatus* extracts. Solvent system: chloroform:ethyl acetate (60:40)

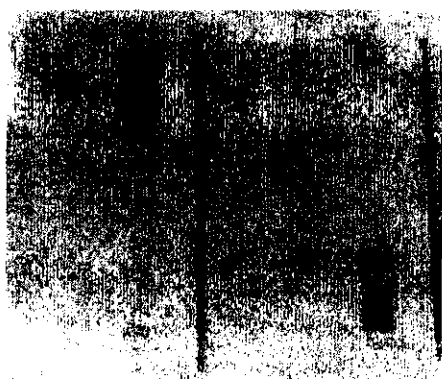


Fig.3 (Left) Thin layer chromatogram of standard β -sitosterol and *O. aristatus* extracts

Solvent: hexane: ethyl acetate (1:1)

(Right) Thin layer chromatogram of standard β -sitosterol and *O. aristatus* extracts

Solvent: hexane: chloroform (1:1)

Regarding the hypoglycemic activity evaluation, mean blood sugar levels of control and treated animals at various time intervals were shown in Figure 4 and Figure 5. From each histogram, the injection of

adrenaline as well as glucose administration significantly induced hyperglycaemia ($p < 0.005$ each) in the control rabbits reaching the maximum level at 1 hr. During this period, aqueous extracts of *O. aristatus* produced a significant inhibition in blood sugar level ($p < 0.05$) in both models. Therefore, the leaf of *O. aristatus* was confirmed to possess hypoglycemic activity.

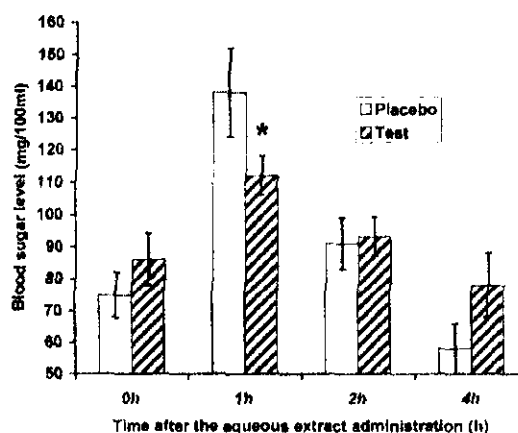


Fig. 4 Time course of the effect of the aqueous extract on adrenaline-induced hyperglycaemia rabbit model

Each bar represents the mean of observations and each vertical bar indicates the standard error of the mean
*denotes ($p < 0.05$)

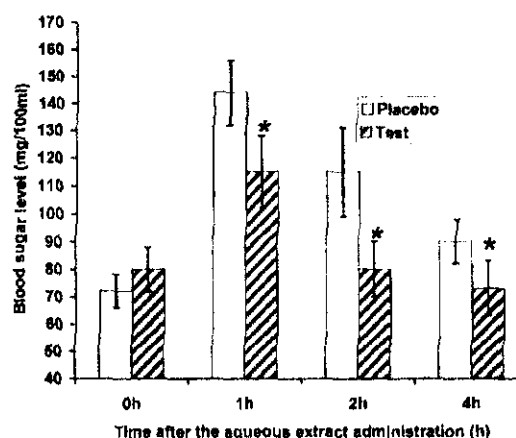


Fig. 5 Time course of the effect of the aqueous extract on glucose-loaded hyperglycaemia rabbit model

Each bar represents the mean of observations and each vertical bar indicates the standard error of the mean
*denotes ($p < 0.05$)

Acute toxicity test indicated no death, in all

animals at doses tested and it was concluded that the median lethal dose (LD50) was more than 8 g/kg when administered orally. Scientific informations on the pharmacological activity of this plant for diuretic activity had been reported by other researchers. The entire plant, except for the roots, is claimed to possess diuretic properties and is used in treating oedema, eruptive fever, influenza, rheumatism, hepatitis, jaundice, urinary and biliary lithiasis [14]. It was also reported that the whole plant contains a bitter glucoside, orthosiphonin, saponins, alkaloids, tannin, essential oil, flavonoids, triterpenoid alcohol, choline, betaine, organic acids (tartaric, citric and glycolic), and potassium salts. Hypoglycemic activity had not yet been reported in the available literatures before this study. Therefore, this experimental finding was the first report for the tested activity and revealed the local name of the plant and its traditionally reputed activity, which were observed to agree in the scientific evaluation on animal model. It was assumed that the tested leaf extract contained one or more hypoglycaemic principles which could significantly reduce the blood glucose levels. As the plant is widely reputed and prescribed for the lowering of blood glucose level in diabetes by the Myanmar people, further clinical research was carried out on healthy volunteers as well as on Type II diabetes mellitus patients (NIDDM) and the results presented at the Myanmar Health Research Congress, 1998.

In the study on healthy Myanmar volunteers, a glucose loaded (75 gm glucose) model was used to induce hyperglycaemic effect. Both the health volunteers and type II diabetes mellitus patients showed a significant blood sugar lowering effect was observed, 1 hr after administration of 175 ml of plant decoction extracted from 25 gm leave of *O. aristatus* when compared to the controls ($p < 0.05$). No evidence of side effect were noted clinically [15,16].

Thus, it can be concluded that *O. aristatus*

possess a hypoglycaemic effect and can be expected to become a useful drug in the management of Type II diabetes mellitus in future.

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Analgesic effect of Chin saw kha thee (*Cydonia cathayensis* Hemsl.) on experimentally induced pain in human subjects

May Aye Than, Mu Mu Sein Myint, Aye Than, Kyi Kyi Myint, San San Myint, Thazin Myint & Tin Nu Swe

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Chin-saw-kha-thee (*Cydonia cathayensis* Hemsl.) from North- East of Shan State of Myanmar is locally claimed to be useful in treatment of gout. In the treatment of gout, there are two types of drugs, one of which lowers the blood uric acid and the other symptomatic drug of anti-inflammatory or analgesic activity. This study aimed to evaluate the therapeutic analgesic efficacy of Chin-saw-kha-thee on experimentally induced cold compressor stimulation pain in healthy subjects. The study was conducted at Clinical Research Unit (Traditional Medicine), Department of Medical Research, Lower Myanmar. The study was a controlled, complete crossover single dose design using aspirin as a positive standard drug. Eighteen clinically healthy volunteers participated in this study and was evaluated on the three basic pain response parameters namely, pain threshold, pain tolerance and pain sensitivity range. The assay was validated by doing a preliminary reproducibility of the pain response parameters (which coefficient of variation of less than $\pm 15\%$ were selected) on the healthy volunteers before the actual study. Both aspirin, 600mg and Chin saw kha thee 10gm (immersed in 150 ml of distilled water for a night) showed significant analgesic efficacy in three parameters ($p < 0.01$ to 0.0005) when compared to placebo (water). No side effects were observed in any of these subjects. From this study it was observed that Chin-saw-kha-thee showed analgesic activity.

INTRODUCTION

The botanical name of Chin-saw-kha is reported to be *Cydonia cathayensis* Hemsl [1]. It is a large squat tree, armed with stout woody spines, $\frac{3}{4}$ inch long cultivated in Kachin villages on account of its fruit [2], leaves glabrous when mature, cordate, serrate, blade 2-4, narrowed into petiole $\frac{1}{4}$ – $\frac{1}{2}$ inch long, stipules leafy, semi cordate, serrate, and stipules cuspidate. Fruit cylindric ovoid or nearly cylindric, 2 inches long, edible, seeds numerous, cultivated in Kachin villages on account of its fruit [2].

The fruit Chin -saw -kha in Myanmar

received from North-East of Shan State of Myanmar, locally claimed to be useful in treatment of gout in the Shan State and Kachin State. Majority of people who are suffering from joint pain and gouty arthritis have been using this plant even though it has never been subjected to scientifically approved trial in human subjects. An acute attack of gout occurs as a result of the inflammatory reaction to crystals of sodium urate (the end product of purine metabolism in human beings). In the treatment of gout, there are two types of drugs, one lowering the blood uric acid by increasing the uric acid excretion or inhibition of uric acid biosynthesis and the other symptomatic

such as anti-inflammatory or analgesic drugs. Recently, another group studied the lowering of blood uric acid efficacy of Chin saw kha thee in human subjects. But the effect was claimed to be not promising. Thus, this trial is attempted to evaluate the therapeutic analgesic efficacy of it in the treatment of moderate to severe pain in human subjects.

Pain is one of the commonest symptom encountered in medical practice and serves the useful function of altering the individual that some component of a physiological system has gone awry. Ideally, pain can be abolished by the removal of the underlying cause, but in many cases, the exact causes of pain is either not easily defined or not readily to remove. Therefore treating pain as a symptom become necessity. Clinical trials in humans involve measurements of pain threshold are; (i) in normal subjects in whom pain has been induced experimentally or (ii) in patients, usually post operative patients, experiencing pain clinically. In this study, measurements of pain threshold on normal subjects in whom pain has been induced experimentally.

MATERIALS AND METHODS

Preparation of Chin saw kha thee

Chin saw kha thee dried fruits, 10 g from North- East of Shan State, were immersed 150ml drinking water of flask for a night (about 12 hours), for each subject. After 12 hours, the cold aqueous extract was filtered through gauge for administration to each subject.

Patient selection

Eighteen healthy volunteers (8 males and 10 females), aged 20 to 45 years, having no history of peptic ulcer, psychiatric problems, alcoholism or abuse of drugs affecting the CNS (analgesic, anxiolytic,

antipsychotics) or drugs taken within the previous 24 hours, were selected after giving a written consent that they were willing to participate in the trial in which they will be receiving repeated painful stimulation and analgesic drugs. This analgesic assay was validated by doing a preliminary reproducibility study of pain response parameters before actual study, (only less than 15% of coefficient of variation) were selected. Only 12 patients were included in actual study.

Experimental design

The main study was a controlled, complete cross-over single dose design where each subjects was randomly assigned by mean of random table to receive either chin-saw -kha thee, placebo (pure water) or aspirin (solution form) as a positive standard drug.

The pain response was assessed 4 times (i.e. each hand half hour apart at 1 and 2 hours after drugs administration) using the method of (7). A wash-out period of at least 48 hours between each study ensured the possibility of carry over effects. The subjects were also instructed not to eat and drink for a period of 90 minutes prior to taking the medication. Cross-over study was carried out by changing the alternative drugs. The pain response was assessed as before. Only 10 total subjects participated completely in the main study.

Trial procedure

Induction of pain was done by asking the subject to place his arm up to the wrist in a luke warm water bath maintained at 35°C. After exactly 2 minutes, the hand was transferred into the ice-water bath saturated with the ice chips and maintained at 0°C and response parameters quantified in seconds.

The subject had been instructed to shout "one" at the slightest sensation that he feels as hurt (pain threshold), but still keeping his hand in the water until it became really distressing and to shout "two", but holding

on until it became totally unbearable and then to shout "three" (pain tolerance) and then quickly withdrew the arm and was put back in the lukewarm water bath until the pain subsided. The times differences between the responses (i.e. 1, 2 & 3) were noted and the difference between pain threshold (PTH) and pain tolerance (PT) was denoted as pain sensitivity range (PSR).

Adverse reactions were recorded if they were observed or volunteered through spontaneous information provided by the subjects and through non suggestive questioning by the observer such as, "Is there anything bothering you"?

Statistical analysis

All results were recorded on standardized forms and analysis was carried out by means of computer. Paired Student's "t" test was done to find out the significance of difference between the pain response parameters of each treatment and the placebo, with ($p < 0.05$) taken as the minimal level of significance.

RESULTS

A total of 10 healthy volunteers (4males and 6 females) took part in the study and their base line demographic and clinical characteristics are shown in table 1. All volunteers gave reproducible response of ($\pm 15\%$) in the 3 basic pain response parameters to be tested.

Table 1. Normal baseline demographic and clinical characteristics of healthy subjects participating in the study

1	Total evaluable subjects	10
2	Sex (males:females)	4:6
3	Mean age (years)	33.9 $\pm$ 1.45
4	Mean height (cm)	146.2 $\pm$ 12.9
5	Mean weight (kg)	46.8 $\pm$ 2.45
6	Mean baseline responses to pain (seconds)	
	- Pain threshold (PTH)	8.4 $\pm$ 1.34
	- Pain tolerance (PT)	41.0 $\pm$ 5.7
	- Pain sensitivity range (PSR)	32.61 $\pm$ 4.8

Table 2 shows the pool mean scores (in seconds) for all response parameters. No significant difference was seen between the base line response and the placebo although some "placebo effect" can be seen in the 3 basic parameters. Chin-saw-kha-thee and aspirin treatments were significantly different from the placebo in all pain response parameters ($p < 0.01$ to $p < 0.0005$). Comparative efficacy between Chin-saw-kha-thee and aspirin treatments showed chin-saw-kha-thee to be superior to aspirin (significantly at severe pain, $p < 0.005$).

Table 2. Pooled means scores (in seconds) for the pain response parameters in healthy subjects

	Onset of pain (pain threshold)	Severe pain	Unbearable pain (pain tolerance)	Pain sensitivity range
Base line	8.4 $\pm$ 1.34	22.6 $\pm$ 3.8	41.0 $\pm$ 5.7	32.61 $\pm$ 4.8
Placebo	10.9 $\pm$ 0.98	28.65 $\pm$ 1.41	44.8 $\pm$ 1.5	33.85 $\pm$ 2.79
Chin-saw-kha-thee	15.4 $\pm$ 1.37 NS	87.41 $\pm$ 12.5* ($p < 0.001$)	135.95 $\pm$ 16.4* ($p < 0.0005$)	120.55 $\pm$ 16.69* ($p < 0.001$)
Aspirin	14.1 $\pm$ 1.53 NS	52.3 $\pm$ 6.77* ($p < 0.01$)	122.5 $\pm$ 16.4* ($p < 0.005$)	108.4 $\pm$ 7.22* ($p < 0.0005$)

Number of subjects (n) = 10

NS = Not significant

* = significant

Figure 1 also shows the pooled mean scores (in seconds) for all the pain response parameters. Figure clearly demonstrate that Chin-saw-kha-thee and aspirin were more effective than placebo treatment. The PT was the most promising parameter for determining sensitive index of analgesic efficacy than PTH.

No adverse effects were noted by the observer as well as complained by the subjects during the study either with Chin-saw-kha-thee or with aspirin.

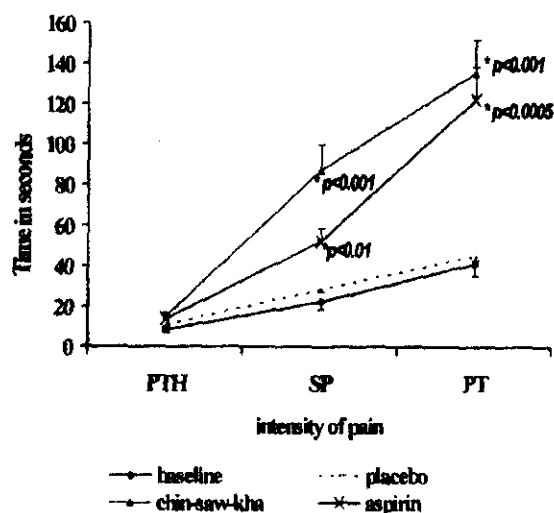


Fig.1. The comparative analgesic efficacy of the chin-saw- kha-thee and aspirin with the placebo treatment and normal base line response

DISCUSSION

The study of traditional medicinal plants and therapeutics play a very important role in health care system of Myanmar because 80 percent of its population are in the rural areas and have been using traditional medicines for centuries. Myanmar people use various herbal medicines tremendously for curing various diseases depending on their own nature and localities. Such medicines may consist of a single potent plant as well as in combination with other potent plants in different ratios by weight or by volumes [3]. Out of many plants some have shown to be the most prescribed analgesic in traditional system for joint pain, gouty arthritic pain and muscular pain. However, none of them have undergone scientific evaluation regarding their analgesic efficacy.

Current techniques for the assessment of pain and its relief were quite diverse, and each method had its advantages [4 & 5]. New drugs are usually screened for analgesic activity in animal model before

they are carried out in humans. But dependable experiment was lacking and various approaches tried out in animal model had been difficult to reproduce [6]. The need for dependable method to test the efficacy of analgesic in man had resulted in many approaches both experimentally and on patients [4 & 5]. The present method was chosen because it has been shown to be the most dependable, least cost and least time-consuming pain model for the analgesic assay in humans [6 & 7].

Since it had been shown that many complex variables such as sex, age, ethnic groups, psychosocial factors, cultural differences etc. [8 & 9], had caused large differences between individuals in their responses to pain and analgesic drugs, a complete cross-over design was chosen to keep the inter-individual variation to the minimum. Successive sessions were also spaced sufficiently far apart so as to minimize the carry-over effects (pharmacological interaction). Aspirin was chosen as a positive control because of its established efficacy and safety [10, 11, 12 & 13] and also because it had been proven to be a comparable standard drug for 3 TMFs (Traditional Medicine Formulations) by the previous study [14].

In this study the findings showed that both Chin-saw-kha-thee and aspirin invariably produced more significant changes in pain tolerance than in pain threshold were in agreement with previous studies which had shown that the pain tolerance was a more sensitive index of analgesic efficacy than pain threshold [7, 14 & 15]. Thus, it was concluded that Chin - saw - kha - thee possessed analgesic efficacy like aspirin.

Chin-saw-kha-thee is easily available locally and cheaply, without significant side effects in their recommended dose, this study justifies their use as alternative to non-narcotic analgesic for the relief of mild to moderate pain.

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Existing practices of general practitioners on diagnosis and treatment of tuberculosis in Yangon

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This study aims to find out the existing practices of general practitioners on management of tuberculosis at general practice (GP) clinics in the selected townships of Yangon. Pre-tested self-administered questionnaires were delivered by hand delivery. Free listing and ranking methods were also done to identify and prioritize the problems encountered in diagnosis and treatment of tuberculosis. Sixty-seven general practitioners in the study (71%) have not experienced any training on current management of tuberculosis yet. It was found that (88%) mentioned Chest X Rays as the most frequent investigation for diagnosis of tuberculosis. Sputum microscopy for Acid Fast Bacilli was expressed by only (9.6%). The most common problem encountered was patient could not afford the cost of drugs to complete treatment. On opinion survey, majority of them pointed out that co-ordination between public health service and general practitioners was essential for better management of TB.

INTRODUCTION

Tuberculosis is a major health problem in the world especially in the developing countries. Moreover, tuberculosis disproportionately affects the South-East-Asia (SEA) Region; the ten countries account for 42 % (1.3 million) of the global burden of reported tuberculosis [1]. Five countries including Myanmar contribute more than 90 % of tuberculosis cases in the region [1]. In Myanmar, tuberculosis is the second priority disease in National Health Plan (NHP, 1996-2001) [2]. The incidence among the general population is about 189 tuberculosis patients per 100,000 populations. Out of which 85 patients are sputum positive cases who are the sources of infection to the community [3]. A cost effective primary health care strategy for tuberculosis control, the DOTS strategy, has been accepted as the strategy for tuberculosis control in all the

member countries of the SEA Region. However, only 15 % of all infectious cases are now being treated with DOTS [4]. The private health sector is the first point of contact for 50 to 80 percent of TB patients in developing countries according to a series of operational research studies conducted by the WHO [4]. In most of the member countries in SEA, more than half of tuberculosis patients are managed by general practitioners. However, collaboration between the private and the public health sectors is weak [4]. Many private practitioners are quite unaware of the WHO or nationally recommended TB treatment guidelines [5]. Therefore, understanding of the existing practices of general practitioners on diagnosis and treatment of TB at general practice (GP) clinics is in urgent need. Moreover, this exploratory study will be a pillar for future action-cum-research on TB control program.

This study was aim to find out existing situation of general practitioners on diagnosis and treatment of TB in selected townships of Yangon.

MATERIALS AND METHOD

It was a cross-sectional descriptive study using both qualitative and quantitative methods.

Among 45 townships of Yangon Division, three townships: Hlaing-thar-yar, Ahlon and North Ok-ka-la-pa, were selected randomly. Based on the assumption of 50% of general practitioners have correct knowledge on management of TB with 95% confidence interval and 5 % precision, the desired sample size was determined as 96.

There were about 50-60 clinics per township on average according to township GP registers. Considering obtaining the coverage of 50 % of the GP clinics in these townships, 30 was required sample size for each township. Among all clinics, those 30 GP clinics were selected randomly.

Pre-testing of self-administered questionnaire was done in South Ok-ka-la-pa Township in order to ensure the quality and feasibility of the study questionnaire as well as to estimate the response rate. Questionnaires were modified accordingly after pre-test. Pre-tested self-administered questionnaire were delivered by hand delivery from clinic to clinic and collected back within a week.

There were three different qualitative methods in this study.

Free listing method was conducted among 17 general practitioners from General Practitioners (GP) section, Myanmar Medical Association (MMA) to identify the problems encountered in diagnosis and treatment of TB at GP clinics. Then, *ranking* was done among those GP to

prioritize the most common problems. *Opinion survey* was also conducted among general practitioners from study areas to elicit opinion and suggestions for better management of TB.

Statistical analysis was done for quantitative data by using Epi-info version 6.0. For qualitative data, matrix analysis was performed. Methodological triangulation was conducted by using different data collection methods. It is aim not only to achieve the objectives of the study but also to capture the complete picture of existing situation of general practitioners on management of TB.

RESULTS

1. Background characteristics

This study was conducted among 94 GP from three townships in Yangon. The ages of the respondents ranged from 25 to 65 years with the median of 35 years. Forty-nine (52%) were male. Only a few, 27 (29%) had the additional training on tuberculosis (TB) conducted as Continuing Medical Education (CME) of GP session, MMA.

2. Existing recording system at GP clinic

It was found that 39 GP (41%) in the study had both patient register books and patient record books. The rest had either one of those recording books. However, 5 GP (5%) had no recording system in their clinic.

3. Reported situation of TB at GP clinics

About one third of the GP (33%) estimated that 30-50 % of TB cases took treatment at GP clinics. Another one third (32%) responded as about 50-80%. Average caseload of TB at GP clinics was wide variation from 1 to 60.

Forty five percent reported that prevalence of TB was more in working age group of 30-45 years and more in male. The common

main complaints of TB cases presented at GP clinics was cough (85%) and followed by fever (72%), loss of weight (51%) and loss of appetite (25%).

4. Diagnosis of TB

Almost all (97%) of the GP performed investigations to confirm the diagnose TB. The most frequent investigation was Chest X-ray (88%). Sputum for AFB was done only by 9% of them.

Most common problems identified by the GP were shown according to their priority ranking order as follows:

1. Most patients could not afford for investigations
2. Limitations in referring patients to public health centre for investigation.
3. Suspect cases lost in follow up call for confirming the diagnosis
4. Patients fail to follow three consecutive sputum examinations
5. Some laboratory results were not reliable
6. Low knowledge on sputum examination which is necessary for diagnosis and treatment monitoring among patients
7. Less accessible laboratory services particularly long distance
8. Some patient could not give previous history of the disease and treatment
9. Time constraint in GP to explain the patients regarding diagnosis

5. Treatment of TB

As a initial treatment for suspected cases at GP, majority (81%) gave antibiotics; some (26%) gave antipyretics and (18%) gave antitussives. Eight GP (8%) reported that they prescribed anti TB drugs without any investigations. The reasons for such practice were:

- signs and symptoms were quite suggestive of pulmonary TB and patients refused to do investigation for various reasons such as unaffordable
- cough with haemoptysis and enlarged

neck glands

- old case with incomplete treatment
- extensive TB in Chest X Ray
- defaulter cases

Seventy GP (74%) prescribed the drug only and instructed to the patients to buy from drug stores. The rest provided the drugs directly.

Duration of the treatment given was varied from 3 to 18 months and 62% said 6 months.

The problems encountered in the treatment were as follows:

1. Patient could not afford the cost of drugs to compete treatment
2. Some patients stopped taking drugs when symptoms were relieved
3. Some took anti TB drugs irregularly
4. Anti TB drugs was not easily available at public health centre
5. Difficult to manage multi-drug resistant TB cases
6. Poor quality of some anti TB drugs
7. Some patients denied the diagnosis and refused to take treatment
8. Difficult to manage TB with other associated diseases e.g. HIV

6. Follow- up

Regarding follow- up of TB cases, 88 GP (93%) reported that they called follow up. The interval of follow up varied from one month to 9 months. Majority (62%) called follow up after one month. The rest GP called follow up daily initially and then monthly depending on treatment category.

Eighty-four respondents (89%) mentioned the problems in follow up. The common problems were:

- Patients felt of symptoms relieved
- could not afford the cost of drugs
- some patients did not come regularly for follow up
- patients came back for follow up only when there were some complaints

Actually majority of the patients took regular follow up only during initial 2 months.

7. Treatment completion

In order to confirm cure and treatment completion, chest X-ray was the most frequent investigation as 89% of the GP responded. Sputum for AFB, blood for CP and ESR were used by about half of them.

Although 67% of the GP decided themselves for treatment completion, the rest 23% referred to specialist to confirm the cure.

8. Referral

Eighty-six GP (91%) referred TB cases for following reasons:

- patient could not afford the cost of drugs
- TB with other complications
- multi-drug resistant TB cases

Majority (91%) referred to Union Tuberculosis Institute (UTI) and 25 (26%) referred to general hospitals. Other 22 (23%) referred to specialist's clinic. The rest stated that the referral place depended on the patient's socio-economic condition i.e. if the patient could afford, they referred to specialist.

9. Common complications of TB

The common complications usually found at GP clinics were haemoptysis, pleural effusion and pneumothorax.

10. Opinions and suggestions

Opinions and suggestions related to public health service

Majority suggested that anti TB drugs should be given by free of charge for all patients. Some expressed as the drugs should be delivered widely with subsidized price for the poor patients. Some said that investigations should be accessible and with reasonable price and free of charge for poor cases.

"It would be more feasible for the patient if all investigation for diagnosis of TB could be done at Township Health Office by free of charge". (38 years old doctor with 8 years government service, 13 years GP service and had not any additional training on TB)

Moreover, some also emphasized that there should be a proper referral pathway and co-ordination between public health service and GP.

"I think it will not be controlled by merely presence of TB campaign in each township. Collaboration and coordination among us is necessary". (29 years old doctor with 2 years government service and 5 years GP service and had not any additional training on TB)

Few GP wanted to get feedback information regarding referred cases. However, only a few stated that necessary support needed for public health centre and not for private clinics.

Opinions and suggestions related to general practitioners

One third of the GP gave suggestion on CME including the ways and means like refresher training and delivering information by pamphlets or leaflets.

"Training in the form of refresher course, case management guidelines and referral system should be given to general practitioners." (35 years old doctor with 6 years GP service and got additional training on TB)

Opinions and suggestions regarding community health education

About 33% of the GP suggested on health education to community as well as to the patients. Information should be emphasized on not only nature and course of the disease but also importance of regularity and complete duration of treatment.

DISCUSSION

In this study, an attempt was made to explore the existing practices of GP in management TB, which is the major area of concern for effective TB control.

It was found that percentage of TB cases took treatment at GP clinics estimated by GP coincided with the estimation of WHO saying that 60-70% of all TB patients in the private sector. As found in many other studies, the GP also stated that prevalence was more in working age group.

Main complaints presented at GP clinics were cough followed by fever and loss of weight, which are early symptoms of TB. This positive finding is important for early diagnosis at GP clinic. However, community awareness on early chest symptoms are still need for further research.

The most important finding which needs to take prompt action was that the diagnosis was made more often based on chest X-ray rather than on sputum microscopy as recommended by NTP and WHO. A few general practitioners (8%) prescribed anti TB drugs without any investigations. These will threaten the magnitude of multi drug resistant problem. These results highlighted that specific instructions or guidelines for management of TB should be delivered to every GP.

One third of the GP suggested that CME for diagnosis, treatment and referral of TB cares. The most common problems encountered in diagnosis and treatment of TB were patient could not afford the cost of drugs and difficulties in referral to public health service. On opinion survey, majority suggested need of free of charge or subsidised price of TB drugs. About one third of the GP gave suggestion for problem in follow-up. Health education should emphasize on course of the disease and importance of regular treatment. Therefore, establishing public/private partner-ship and

collaboration in management of TB will solve these problems.

Since this was an exploratory small-scale study, then the findings could not be representative for the whole country. There were also some limitations of data collection tools such as using self-administered questionnaire. Observation and record review could not be done. However, conducting methodological triangulation by using different data collection methods such as free listing and ranking, it could capture the picture of existing practices of general practitioners on management of TB.

Recommendations

In the light of above findings, the following recommendations could be made.

- to give effective training on TB to ensure to reach for every GP
- to establish public/private partnership on management of TB for more effective TB control
- to deliver health messages regarding TB especially and taking regular and complete treatment.

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Purgative effect of Pway-mezali (*Cassia alata* Linn.) leaves on healthy subjects

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Majority of Myanmar national people lived in rural areas and have been using traditional medicinal plants for hundred of years. Many people have used some medicinal plants, which claimed to have purgative activity, as crude drugs or as traditional medicine formulations. With the aim to evaluate the therapeutic purgative efficacy of *Cassia alata* Linn. (Mezali-gyi or Pway-mezali), on healthy subjects was conducted at Clinical Research Unit (Traditional Medicine) Department of Medical Research, Lower Myanmar. The study was a controlled, complete cross-over single dose design using magnesium sulphate and phenolphthalein as positive standard. Ten clinically healthy volunteers were participated in this study. Pway-mezali fresh leaves 4 g which was just heated for few seconds for softening, magnesium sulphate 1 teaspoonful, and phenolphthalein 0.8 g and placebo 2g were administered weekly. Pway-mezali, magnesium sulphate and phenolphthalein showed significant ($p < 0.00005$) purgative efficacy on frequency and volume when compared to placebo. Pway-mezali fresh leaves showed no significant different efficacy on frequency, volume and onset when compared to phenolphthalein. It was also found that the onset time of action of Pway-mezali and phenolphthalein is prolong than magnesium sulphate due to the different mechanism of action. No side effects were observed in any of these subjects. It was concluded that Pway -mezali leaves showed purgative activity.

INTRODUCTION

The use of plants based products for disease prevention and treatment has become increasingly popular in ASEAN countries. World Health Organization currently encourages, recommends and promotes traditional/herbal remedies in National Health Care Program because such drugs are easily available at low cost, comparatively safe and the people have faith in such remedies.

Myanmar is rich in varieties of medicinal as well as aromatic plants due to the presence of different climate zones in the country. There are 7000 different known plants growing in Myanmar and the most of them have been recognized as medicinal plants

[1]. Myanmar herbal medicine has been practiced for about 2500 years. Myanmar people use herbal medicine tremendously for curing various diseases. The study of traditional medicinal plants and therapeutics play a very important role in health care system of Myanmar because 80 percent of its population are in the rural areas and have been using traditional medicine for centuries. The Government of Myanmar has urged to enhance the quality and to promote the systematic development of traditional medicine, which is of paramount importance in ensuring the health and well being of Myanmar people.

There are numerous indigenous plants, which are reputed to be effective for purgative activity. Pway-mezali is easily

available throughout in our country. Pway-mezali (family-Leguminosae) is three meter tall. Leaves are pinnately compound 30-40 cm long with pairs of broad oblong leaflets, blunt at the tip, unequal at base, the terminal pair much larger, about 15 cm long and 8cm wide [2 & 3]. It is one of the plants claimed to treat for ring worm and purgative locally [4].

Preliminary requisite, acute toxicity test was done on mice model is mandatory. No acute toxicity was seen at 8g/kg (the highest permissible dose). The present study, is therefore, aimed to prove the effect of Pway-mezali, scientifically, for safety of people.

OBJECTIVES

General objectives

To compare the effect of *Cassia alata* L. (Pway-mezali) with other known purgative in healthy subjects

Specific objectives

- To find out whether Pway-mezali have the purgative activity, on healthy subjects.
- If so to compare with the efficacy with that of the standard magnesium sulphate and phenolphthalein.

MATERIALS AND METHODS

Collection of medicinal plants

The specimens used in this study were collected from the Department of Medical Research, Lower Myanmar, Yangon. The plants were identified according to the description given in the characterization literatures regarding taxonomical [5, 6, 7 & 8]. The collection time of the leaves was between November to January after the rainy season and full-grown leaves of nearly uniform size were plucked from the branches.

Preparation of Pway-mezali

The fresh leaves were weighed of 4g and

cleaned with water and then just heated on the hot plate for few seconds for soft and wilt the leaves to prevent the greenly taste. It was one of the traditional use in rural area. These leaves were readily to ingest for study subjects.

Patients selection

Ten healthy volunteers (4 males and 6 females) age between 18 to 45 years, having no history of taking purgative within 1 weeks, were selected after giving a written inform consent that they were willing to participate in the trial. Those who had acute diarrhoea within one week with or without taking anti diarrhoeal drugs were excluded.

Experimental design

The main study was a controlled, complete crossover single dose design where each subjects was randomly assigned by mean of random table to receive either Pway-mezali (4g), placebo (2g), magnesium sulphate (1 teaspoonful i.e. approximately 2g) or phenolphthalein (0.8g).

Trials procedure

All selected patients were explained about the clinical trial, its objectives and procedure. The subject were also instructed not to eat and drink for a period of 2 hours prior to taking the medication and 30 minutes after the medication. A wash-out period of at least one week between each study. Cross-over study was carried out by changing the alternative drugs. The purgation response was recorded as frequency, time of onset, volume and form of stool of each time, up to 24 hours. Adverse reactions were recorded if they were observed or volunteered through spontaneous information provided by the subjects and through non-suggestive questioning by the observer such as, "Is there anything bothering you."

Statistical analysis

All results were recorded on standardized

forms and analysis was carried out by means of computer. Paired Student's "t" test was done to find out the significance of difference between the purgative response of each treatment and placebo, with ($p < 0.05$) taken as the minimal level of significance.

RESULTS

A total of 10 healthy volunteers (4 males and 6 females), participated in this study and their baseline demographic and clinical characteristics are shown in table 1. Seven out of 10 have constipated for few days and the rest had regular daily motion.

Table 1. Normal baseline demographic and clinical characteristics of healthy subjects participating in the study

1	Total evaluable subjects	10
2	Sex (males : females)	4 : 6
3	Mean age (years)	36 ± 3.1
4	Mean height (cm)	152.7 ± 1.8
5	Mean weight (kg)	47.6 ± 2.4
6	History of constipation within 48 hours	7 subjects
7	Past history of taken purgative	10 subjects

Table 2. Comparison different purgative in frequency, times of onset and volume of passed stool in healthy subjects

	Frequency of passed stool (in numbers)	Onset of purgation (in hours)	Volume of passed stool (in liter)
Placebo	0.6 ± 0.22	10.15 ± 3.41	0.08 ± 27.1
Pway-mezali,	3.1 $\pm 0.37^{**}$	5.8 $\pm 0.51^*$	0.73 $\pm 98.4^{**}$
Magnesium sulphate	4.6 $\pm 0.49^{**}$	2.2 $\pm 0.18^*$	1.125 $\pm 100.4^{**}$
Phenolphthalein	3.5 $\pm 0.26^{**}$	4.7 $\pm 0.71^*$	0.79 $\pm 94.8^{**}$

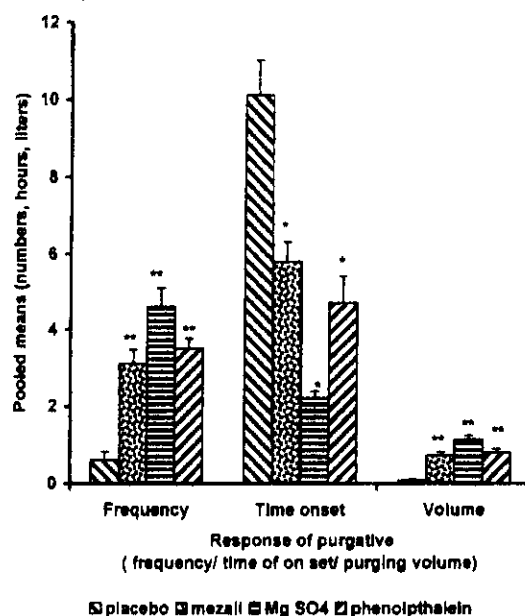
Number of subjects (n) = 10

* = $p < 0.05$

** = $p < 0.0005$

Table 2 shows the comparison of response for purgation to Pway-mezali, placebo, magnesium sulphate and phenolphthalein. The frequency, volume and on set of purgation of Pway-mezali, magnesium sulphate and phenolphthalein were significantly different from the placebo treatment ($p < 0.05$ - $p < 0.0005$).

The comparative efficacy (frequency, volume and on set of purgation) between Pway-mezali, and Phenolphthalein treatments showed not significant difference. The comparative efficacy (frequency, volume and on set of purgation) between Pway-mezali, phenolphthalein and magnesium sulphate showed magnesium sulphate to be superior to Pway-mezali and phenolphthalein ($p < 0.05$ - $p < 0.0005$).



*= $p < 0.05$ **= $p < 0.0005$

Fig 1. Comparison of effectiveness of different purgatives in frequency, times of onset and volume of passed stool in healthy subjects.

Figure 1 shows comparison between effectiveness of different purgative in frequency, times of onset and volume. These figures clearly demonstrated that Pway-mezali, phenolphthalein and magnesium sulphate were significantly different from the placebo treatment ($p < 0.05$ & $p < 0.0005$) but statistically difference not

only between Pway-mezali, and magnesium sulphate but also phenolphthalein and magnesium sulphate.

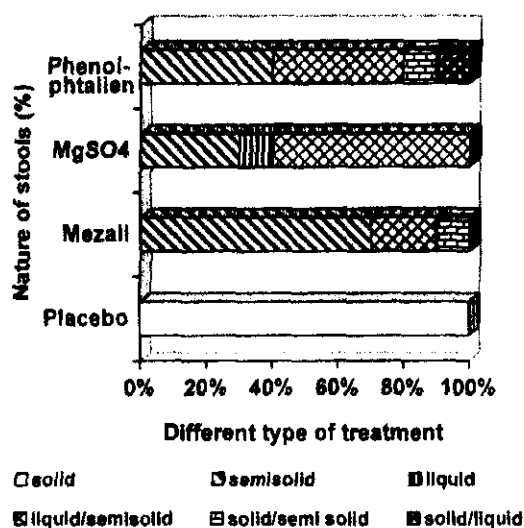


Fig 2. Comparison of nature of stool in different type of treatments

Figure 2 shows comparison of nature of stool in different treatment groups. There was unformed stool passed during the study period in all type of purgative, more liquid stool in magnesium sulphate and only solid stool in placebo treatment.

DISCUSSION

In Myanmar, most the people of constipation is treated self medication with traditional medicine such as tay-hsay, wun-hnoke-hsay, pway-kaing-hsay, and some herbs like Pway-mezali flowers, leaves, and Say-pa-lei leaves. Some people took modern purgative like magnesium sulphate and phenolphthalein. Because of they believed that "Make sure habit of regular motion prevent problem of headache" (ဝမ်းတစ်လုံးကောင်းရင် ခေါင်းမခဲဘူး). In some people having piles, they have taken these leaves/flowers to pass unformed stool (semisolid stool) for preventing the protrusion and bleeding of piles during strain in defecation.

This study showed that Pway-mezali was

effective by increasing frequency purging volume, and passing of unformed stool (soft and semi-fluid stool). The efficacy of Pway-mezali is approximately equal to that of phenolphthalein which is stimulant purgative. It's mechanism is to increase the intestinal motility causing decreased absorption of salt and water secondary to decreased transit time [9]. magnesium sulphate was effective by increasing the passed stool as most liquid due to its' mechanism of action. magnesium sulphate caused retention of fluid in colonic contents, there by increasing bulk and softness and facilitating transit because of their hydrophilic or osmotic properties [9]. But all drugs, did not produce any significant adverse effect of discomfort which required treatment.

According to chemical studies, it contained anthraquinone, Sitosterol, chrysophanol glycoside, phytosterol, tannin, and deoxycoelin [2]. The pur-gative activity of *Cassia alata* had been reported by Thamlikitkul [10] in 1990. Eighty three percent passed stool in 24 hours, when treated with *Cassia alata* on at least 72 hours constipated patients. Pway-mezali is available locally and cheaply, and thus, may prove beneficial for the treatment of constipation.

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Experiences and knowledge of malaria among hospitalised patients of Myanmar

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A study was conducted among hospitalised patients from General Hospital of Mingalardon. The aim of the study was to identify the malaria related perceptions, beliefs and health seeking behaviours among hospitalised patients so that necessary health education may be given effectively. A standardised questionnaire was administered to 300 hospitalised medical and surgical patients from the hospital. The respondents were all male with majority between 18 and 55 years of age. It was found that misconception on causes of malaria included: eating banana and fruit 39.71%; drinking swampy stream water 60.3%; tiredness 29.7%; sleeplessness 34.3%; changing weather 32.3%; and getting caught in the rain 34.3%. As for malaria preventative measures, the study found that 68% of them do not want to use mosquito net owing to their habitual nature. In combating malaria, prevention is the most efficient method to be employed. Once it is contracted, effective cure of the disease can only be realized by employing correct use of the anti-malaria drugs with full confidence and reliance. The study explored and determined the experience of malaria in hospitalised patients and their beliefs and practices in combating the disease. The results of the study can be used to identify appropriate approaches and methods to give the patients the broader knowledge in malaria and to educate them in the self-care health practices to prevent and combat malaria effectively. The findings suggest that the strong measures should be taken to further improve the level of knowledge and conception of the patients in malaria.

INTRODUCTION

Malaria is one of the top communicable diseases that has largely affected the health and social well-being of the world populace. It can directly affect the physical health of the people and also indirectly influence the psychological health status. Concerted efforts at national level as well as regional and global levels have been made continuously and attentively to combated this troublesome disease without much success.

It is also a problematic disease in Myanmar. Myanmar is one of the malaria endemic places in the world owing to its climatic and geographic situations, which provide

encouraging ground for breeding of mosquitoes and cause difficulties for people struggle against the disease. The most troublesome areas in the country are located mainly in the borders with forested mountain regions where mosquitoes can bred well; hence the transmission of malaria can be greatly caused by those creatures acting as vectors. Border areas in Myanmar are mostly forest-fringe foothill and swamp areas where temperature and rainfall would most likely allow the malaria mosquitoes to survive and multiply.

As chances of contracting malaria by people are very high, positive measures should be effectively taken to prevent them from being stricken by the disease.

Justification

The National Surveillance Vector Borne Disease Control Program judged malaria as a top communicable disease in Myanmar in 1997. There are many factors influencing the morbidity of the disease. While many of them such as geographical situation, bio-environment and weather conditions cannot be easily dealt with in controlling malaria, preventive measure and self-care health practices can be effectively used in control activities against the disease.

Win, *et. al.* (1993) concluded that the level of malaria related knowledge among the general population in Myanmar was quite low in spite of extensive campaign on malaria control activities. Although the extensive campaigns on malaria control were launched and health education and information related to the disease were provided to the public, large portion of the local populace still don't recognize the value of self-care practices in combating the disease. It was also recommended that an in-depth research on socio-behavioural ethno-physiology of Myanmar people should be carried out so that malaria control could be supported by an extensive effort on changes of behavioural patterns.

Perception of the populace regarding the natural pattern of transmission, clinical manifestations, severity and controllability

of malaria, as well as their notions about their susceptibility to it has been deemed to be associated not only with the prevalence of the diseases, but also with the population's acceptance and participation in control measures.

It is apparent that preventive measures and self-care practices could result in effective control of malaria. It is therefore considered that the perspective in malaria and health seeking behaviours among the community are necessary to assess in order to implement effective methods and approach in combating malaria.

Myanmar has the total population of 50 millions of which 31% live in high-risk area, 28% in moderate-risk area, 20% in low-risk area and 21% in no-risk area with regard to malaria infection.

According to health statistics, malaria morbidity rates (1992-2000) were recorded as follows:

- Average number of clinically diagnosed malaria cases - 600 thousands cases per year (range 568,000 – 702,000)
- Malaria morbidity rate - 11.7 per 1000 population
- Proportion of malaria cases (outpatient department) to total patient attended – 10% (state and division wise range 2.4% - 26.4%)

Table 1. Malaria morbidity and malaria mortality rate (1992-1997)

Year	No. of clinical malaria (OP)	% of total (OP)	No. of clinical malaria (IP)	% of total (IP)	No of malaria deaths	Case fatality rate	Deaths per 100,000 population
1992	657	7.6	132	19.2	4739	3.6	11.2
1993	573	7.1	129	18.9	4219	3.3	9.8
1994	565	7.8	132	19.0	4380	3.3	9.9
1995	551	8.9	105	17.2	3744	3.5	8.4
1996	561	10.0	103	17.1	3424	3.3	7.5
1997	480	9.5	88	15	2943	3.4	6.3

- Proportion of malaria cases (inpatient department) to total patient admitted – 15.9% (range 5.4% - 23%)[2].

During 1997, twelve epidemics were recorded and most of these epidemics were related to development projects and new settlement activities.

Although total number of malaria cases was declined due to changing health-seeking behaviour, proportion of malaria cases in outpatient department became increased.

Malaria mortality (1992-2000) were found as follows:

- Number of reported malaria deaths – 2748 (2000)
- Range 2748-4400 (1992- 2000)
- Malaria mortality rate – 5.5 per 100,000 population (2000)
- Case fatality rate – 3.3%

Although average number of reported malaria deaths and malaria mortality rate were declined, case fatality rate was more or less stable at about 3.5% [3].

Malaria control activities have been carried out by coordination and cooperation among the various organisations. It consists of malaria staff, basic health staff, voluntary health workers, NGOs, health related ministries and the private sectors. Special Care Group is mainly responsible for planning, monitoring, supervision, quality assessment, evaluation, training, applied field studies and technical guidance [4].

As malaria is still a major public health problem in Myanmar, it is also the major objective of the country's health programme to control malaria in an effective way.

Objectives

- To assess the malaria knowledge in hospital setting;
- To explore the malaria experiences in curative and preventive measure;

- To identify the health seeking behaviours of hospitalised patients in malaria;
- To determine the conception of hospitalised patients in malaria, which can be corrected with appropriate health education.

MATERIALS AND METHODS

Cross sectional descriptive design with quantitative methodology was employed to study a selected group of male hospitalised patients from General Hospital of Mingalardon Township in Yangon Division. The data were collected using simple random sampling method from 15th October to 15th November 2000. They were interviewed by the interviewer and asked to answer the questionnaires individually. There were 60 items in the questionnaires consisting of 20 perception items, 20 beliefs and 20 health seeking behaviours. The questionnaires were constructed based on various malaria related health information from Ministry of Health, Department of Health, Vector Borne Control Section and World Health Organization, Malaria Control Information. Pretesting was done to test the questionnaire was carried out on thirty male staff from medical corps.

The target population was 300 hospitalised medical and surgical patients from General Hospital of Mingalardon Township in Yangon Division.

Method of Analysis

Data were coded and transferred into coding sheets and processed by the assistance of a personal computer using SPSS/PC+ Software. The mean score percentage was calculated for each level of age and educational status.

Before the questionnaire was administered, an interview was done to reduce the anxiety and to establish personnel rapport in order to obtain correct and valid data.

RESULTS AND DISCUSSION

The respondents were all male with majority between 18 and 50 years of age and educational level of 34.3% with high school level and above, 65.7% with below high school. Although the background level of education is not directly related to health knowledge, it is one of the supportive measures to learn health care practices in malaria.

It was found that misconception on causes of malaria include: eating banana and fruits (39.71%); drinking swampy stream water (60.3%); tiredness (29.7%); sleeplessness (34.3%); changing weather (32.3%); and getting caught in the rain (34.3%).

Table 2. Theme of perceptions, beliefs and practices on malaria findings

Perceptions and beliefs on causes of malaria	%	Practices	%
Eating banana and fruits	39.71	Respondents do not want to use mosquito net due to the heat inside the net	43
Drinking swampy stream water	60.3	Due to feeling of airlessness inside the net	32.3
Tiredness	29.7	Habitual nature (sleeping without net)	68
Sleeplessness	34.3	Traditional medicines in local pharmacy are used	>50
Changing weather	32.3	Various health seeking behaviours are taken	>50
Getting caught in the rain	34.3		

As for malaria preventive measure, the study found that 43% of respondents do not want to use a mosquito net because they couldn't bear the heat inside it especially in the warm weather condition, 32.3% do not want to use a mosquito net due to feeling of airlessness

inside it and 68% of them do not want to use a mosquito net owing to their habitual nature.

According to the study, the level of knowledge in malaria among the patients can be considered as below par and their belief on causes of malaria was the most disappointing fact among all. None of the respondents answered mosquito-biting as the cause of malaria. Although background education is not directly related to health knowledge in malaria, some background education can help learning health-care practises. According to the study, most of the respondents' levels of education are below high school standard.

Although the patients were administered anti-malaria drugs regularly for their malaria illness during the hospitalisation, most of the respondents used various health curative measures. It was found that some of the patients did not take the full dosage of medicine but kept them with the intention of using them again at next relapse.

Although many people practise self-treatment of malaria with drugs available at stores and pharmacies, very few percentage of them aware of the most effective drugs and correct dosage of them. Many of the sellers of pharmacy are found to be non-medical personal. An educational campaign directed at correcting some of these misconceptions should result in more effective self-care practices on greater acceptance of personal protection methods and vector control and drug treatment programs. More than 50% of the respondent's answer that traditional medicines in local pharmacy are used because they are readily available and inexpensive than western medicines. More than 50% of the respondents take various health seeking behaviours; traditional healers, friends or peer group pressure; senior citizen's guidelines.

This study recognized that reasonably large number of respondents does not have correct

knowledge on causes of malaria and some of them have misconception on it. Their view on preventive measure and curative methods varied indifferently and practices on incompetent method of traditional treatment were also evident.

The level of knowledge on signs and symptoms of malaria are reasonably acceptable. More than 50% of the respondents recognize the high body temperature, cold and clammy, chills and rigors, aches and pain in the whole body, severe headache and sweating as signs and symptoms of malaria. But they cannot mention them in correct sequential order.

Although the majority of the respondents recognize the use of western medicine for curing malaria, they do not have proper knowledge on drugs used for treating malaria. They often use them on irregular basis with incomplete dosage resulting ineffective cure for the disease. It has long been recognized that health knowledge on malaria related drugs carried some significant weight in controlling malaria.

The preventive measures for malaria are also ineffective due to misconception by the people on causes of malaria and their

traditional habits. They feel uneasy to sleep with the mosquito nets because of airlessness and warmth inside it. Therefore, it was concluded that measures should be taken to further improve the level of knowledge and conception of the people. New strategy should also be adopted in providing health education and to initiate behavioural change in hospitalised patients.

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Safety and efficacy of coloured liquid Russell's viper (*Daboia russellii siamensis*) antivenom manufactured by Myanmar Pharmaceutical Factory

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Mono specific liquid Russell's viper (*Daboia russellii siamensis*) antivenom manufactured by Myanmar Pharmaceutical Factory (MPF) is clear and colourless. However, some recent batches of liquid antivenom have tinge brown colour which raises concern about its safety in treating Russell's viper bite cases. In order to determine its efficacy and safety, which is the objective of the study, potency assay and testing of pyrogenic reactions of the antivenom were carried out. Neutralisation of different biological activities of the Russell's viper venom by four batches of coloured antivenom was determined by WHO recommended methods. Pyrogenic testing of the antivenom was carried out on rabbit model. Results indicated that there was batch-to-batch variation in neutralising efficacy of the antivenom and 2/4 batches tested were pyrogenic. A similar observation of variable potency of MPF mono specific antivenom and development of pyrogenic reactions following antivenom therapy in Russell's viper bite cases have been documented. Since it possess venom neutralising efficacy, it could be used for treating Russell's viper bite cases. However, because of its variable efficacy, clinical trial and dose finding experiments of the antivenom need to be carried out since these haven't been studied yet.

INTRODUCTION

Snakebite is an occupational hazard of our farmers. The morbidity rate of snakebite based on 100 hospital returns (1998-2000) is 5024 (3862-5830) and mortality rate 5.6% (5.2-5.95) [1]. The main stay of management of snakebite is early administration of specific potent antivenom for specific bite. Myanmar Pharmaceutical factory (MPF) manufactures enzyme refined liquid and lyophilized mono specific antivenom for treating specific bite through out the country. National requirement of antivenom is on rising trend. The liquid preparation of antivenom is clear and colorless. Recently, some batches of MPF liquid mono specific Russell's viper (*Daboia russellii siamensis*) antivenom are coloured which raises concern about its safety and efficacy for use in management of specific bites. The

objective of the study is to determine safety and potency of the antivenom by determination of its pyrogenicity and neutralising efficacy to different biological properties of Russell's viper venom and to give a suitable recommendation for its use in management of Russell's viper bite cases.

MATERIALS AND METHODS

Materials

Venom

Pooled Russell's viper (*Daboia russellii siamensis*) venom collected from Thara-waddy (n=14, length>90cm), Bago Division was used in the experiment.

Antivenom

Four batches of liquid Russell's viper antivenom (MPF) D 01085, date of

manufacture (DOM) 8-5-01, F 01088, DOM 20-6-01, G 01091, DOM 24-7-01 and G 01093, DOM 15-8-01 were available for potency assay and testing for pyrogenicity. Reference lyophilized Russell's viper antivenom DH95758, DOM 201195 and two batches of monospecific liquid antivenom F 96770, DOM 22796 and C 98011, DOM 30498 were included in the study.

Methods

Characterisation of the Russell's viper venom

Characterisation of biological properties of the pooled Russell's viper (*D.r.siamensis*) venom: LD₅₀ i.v., coagulant, necrotic, haemorrhagic, defibrinogenating and capillary permeability increasing activities was carried out using WHO recommended techniques [2].

Potency assay of antivenom

Neutralisation of the Russell's viper venom by antivenom

Neutralisation of different biological properties of the Russell's viper venom: lethality (5LD₅₀ i.v.), coagulant (10MCD), haemorrhagic (3MHD), necrotic (3MND), defibrinogenating (5MDD) and capillary permeability increasing (100 MCPID) activities was carried out by WHO recommended standard test of neutralising activity [2].

Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, incubated at 37°C for 30 min. and injected into laboratory animals. The end point is taken as the minimum amount of antivenom required to neutralise 50% of the biological effects of venom. For determination of neutralisation of lethal activity ED₅₀ (effective neutralising dose) is used. It is the minimum amount of antivenom that will save 50% of the test animals in 24h following the injection.

Pyrogen reactions testing

Preliminary screening of the antivenom for pyrogen reactions was carried out using 3 rabbits per sample and confirmatory test on 5 rabbits per sample.

Method

Five mls of liquid antivenom (<10ml per Kg) was given intravenously into ear vein of each rabbit in 10 minutes and rectal temperature was recorded at one, two and three hours intervals following injection. The control temperature of each rabbit is determined within 30 minutes prior to testing. The rabbits are conditioned for 7 days before testing [3].

Interpretations

If no rabbit shows an individual rise in temperature of 0.6°C or more above its respective control temperature, or if the sum of the three individual maximum temperature rise does not exceed 1.4°C, the product is labeled as pyrogen negative. If the results exceed above specific criteria, then the product is retested using 5 other rabbits. If not more than 3/8 rabbits show individual rise in temperature of 0.6°C or more and if the sum of the 8 individual maximum temperature rises does not exceed 3.7°C, the product is labeled as pyrogen negative.

RESULTS

Biological properties of the Russell's viper venom

Biological properties of the pooled Russell's viper venom are: MHD (21.08µg/rat), MND (19.9µg/rat), MDD (1.2µg/mouse), MCPID (0.0036ug), LD_{50iv} (2.34µg/mouse) and MCD (1.256µg/ml).

Table. Neutralization of different biological properties of the Russell's viper venom by antivenom

Batch	Prep	Colour	3MHD	3MND	100MCPID	3MDD	5LD _{50iv}	10MCD
			63.24µg/rat	59.7µg/rat	0.36µg	6µg/mouse	11.7µg/mouse	12.56µg/ml
F96770	lq	Colourless	12.5	25	5	2.5	5	0.078
C98011	lq	Colourless	40	20	1.25	5	20	0.3125
D01085	lq	brown	5	20	10	5	10	1.25
F01088	lq	brown	7.4	40	5	2.5	20	2.5
G01091	lq	brown	3.75	20	1.25	2.5	10	1.25
G01093	lq	brown	20	40	0.625	2.5	10	2.5
DH95758	lyd/ref	Colourless	10	5	0.78	3	5	0.0625

prep = preparation

lq = liquid

lyd = lyophilised

ref = reference

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MCPID = Minimum capillary permeability increasing dose

MDD = Minimum defibrinogenating dose

LD_{50iv} = Median lethal dose

MCD = Minimum coagulant dose

Potency assay of the antivenom

Results of neutralization of different biological properties of Russell's viper venom by the antivenom are shown in the table.

Colour of antivenom

The colour of the 4 batches of antivenom (D 01085, F 01088, G 01091, G 01093) is tinge brown and that of the other liquid antivenom and reconstituted antivenom is clear and colourless.

Potency assay

Neutralisation of different biological properties of the Russell's viper venom by the coloured and colourless liquid antivenom is comparable except the former is 2-3 times more potent than the latter in neutralising haemorrhagic activity and 20-40 times less potent than the latter in

neutralising the coagulant activity of the venom.

On comparing the neutralising efficacy of coloured with the reference antivenom, the former is 4 times more potent than the latter in neutralising haemorrhagic activity. However, it is less effective in neutralising lethal (2-4 times), necrotic (4-8 times), coagulant (20-40 times) and capillary permeability increasing activity (8-128 times).

Batch-to-batch variation in neutralising efficacy of the biological properties of the venom is observed in both coloured and colourless antivenom.

Pyrogen reactions testing

Pyrogenic reactions were positive in 2/4 batches of the coloured antivenom tested (data not shown). It is not tested in other liquid and lyophilized antivenoms.

DISCUSSION

The four batches of monospecific liquid Russell's viper antivenom tested for its safety and potency have tinge brown colour and possess variable efficacy in neutralising different biological activities of the Russell's viper venom as in earlier liquid antivenom. A similar batch-to-batch variation in neutralising efficacy of clear colourless liquid and lyophilized monospecific Russell's viper antivenom has been reported [4]. The tinge colour of the antivenom does not indicate loosing of its efficacy. Denatured antivenom turns cloudy and precipitates out [5].

Two of the four-coloured antivenom tested is pyrogenic. In our earlier clinical studies on antivenom response in Russell's viper bite cases admitted to various hospitals, 50% of the antivenom treated cases have pyrogenic reactions characterized by chills and rigor and shaking of the patient's bed [6]. It is envisaged that the pyrogenicity of the coloured antivenom is comparable to that present in colourless liquid antivenom used in treating Russell's viper bite cases.

Since the coloured antivenom (1ml neutralises 1 mg venom) are 20-40 times less potent in neutralising coagulant property of the Russell's viper venom, it is expected that more antivenom will be required to correct venom induced coagulant defect in Russell's viper bite cases. Clot restoration occurs 6h following administration of 4 ampoules of earlier batches of antivenom which have neutralising efficacy of 1ml to 2 mg venom [6]. However, in one systemic envenomed patient admitted to Yaekyi hospital, clot restoration returned 18h following administration of ten ampoules of the antivenom [7]. Such variation in antivenom efficacy could be improved by using widely pooled potent antigen in raising antivenom [8].

Since antivenom is expensive to produce and the coloured antivenom possess variable neutralising potency, it could be used for treating Russell's viper bite cases as in colourless antivenom, however because of its variable efficacy, it is suggested that dose finding clinical trials of the antivenom should be carried out since these have not been studied yet.

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Stability of potency of Russell's viper (*Daboia russelii siamensis*) antivenom on storage

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Monospecific liquid Russell's viper (*Daboia russelii siamensis*) antivenom manufactured by Myanmar Pharmaceutical Factory is used for treating specific bites through out the country. Because of variable responses of the antivenom in correcting venom-induced effects in Russell's viper bite cases, possibility of within batch variation and stability of its potency on storage are considered. The study attempts to verify the assumptions. Neutralisation of biological activities of the Russell's viper (*Daboia russelii siamensis*) venom by five samples of a batch of mono specific liquid Russell's viper antivenom, C 98011, date of manufacture 30-4-98, date of expiry 30-4-01 were studied by WHO recommended methods at 7-9 month and 13-15 month following manufacture. Results indicate that there is within batch variation in neutralization of different biological activities of the venom by the antivenom. There is reduction in neutralizing efficacy of necrotic and capillary permeability increasing activities of the venom by the antivenom at 13-15 month following manufacture compared to that of 7-9 month. Study suggests that more antivenom would be needed to neutralise necrotic and capillary permeability increasing activities of the venom when antivenom aged. Within batch variation and decrease in potency of aged antivenom could be contributing factors accounting for variable responses of the antivenom in correcting some venom-induced effects seen in Russell' viper bite cases.

INTRODUCTION

Russell's viper bite is an occupational hazard of our farmers. Monospecific (both liquid and lyophilized) Russell's viper (*Daboia russelii siamensis*) antivenom manufactured by Myanmar Pharmaceutical Factory (MPF) is used for treating specific snakebite cases. It was found that MPF monospecific Russell's viper antivenom has better neutralising potency than that of foreign antivenom such as Thai Red Cross and Indian SII and Barat Serum Vaccines [1]. It is well established that liquid antivenom stored at different temperatures suffer different rates of activity loss [2-4]. Liquid antivenom stores at 4°C will keep its potency up to the time of expiry (up to 3 years). Earlier clinical studies indicated that antivenom used in treating systemic envenomed Russell's viper bite cases failed

to correct some venom induced effects such as pro coagulant activity [5] which could be due to batch-to-batch variation of antivenom [6], however, possibility of within batch variation and stability of antivenom on storage could not be ruled out. The study attempts to verify these assumptions.

MATERIALS AND METHODS

Pooled Russell's viper venom collected from Tharawaddy (n=14, length >90cm), Bago Division was used in the experiment. Five samples of a batch of MPF monospecific liquid Russell's viper antivenom, batch no. C98011, date of manufacture 20-4-98, date of expiry 30-4-01 were available for potency testing. Samples are aliquoted, stored at 4°C and discarded after use.

Table 1. Potency assays of the five samples of a batch of antivenom (C 98011) at 7-9 month and 13-15 month following manufacture

Code	ASV age (mth)	3MHD 63.24µg/rat	3MND 59.7µg/ rat	100MCPID 0.36µg	5MDD 6µg/ mouse	5LD _{50iv} 11.7µg/ mouse	10MCD 12.56µg/ ml
B	7	100 µl	20 µl	5 µl	10 µl	20 µl	0.3125 µl
	13	100	100	5	5	10	0.3125
A	8	50	25	10	5	20	0.3125
	14	50	50	10	5	20	0.3125
C	9	50	12.5	5	5	10	0.625
	15	50	50	10	5	10	0.625
D	9	100	25	5	2.5	5	0.625
	15	100	50	20	5	10	1.25
E	9	100	50	5	2.5	5	1.25
	15	100	50	10	5	10	1.25

mth = Month

ASV = antivenom

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MCPID = Minimum capillary permeability increasing dose

MDD = Minimum defibrinogenating dose

LD_{50iv} = Median lethal dose

MCD = Minimum coagulant dose

Characterisation of venom

Biological properties of the pooled Russell's viper (*D. r. siamensis*) venom: LD₅₀ i.v., coagulant, necrotic, haemorrhagic, defibrinogenating and capillary permeability increasing activities were determined by WHO recommended techniques [7].

Neutralisation of the Russell's viper venom by antivenom

Potency assay of five samples of antivenom was carried out at 7-9 month and 13-15 month following manufacture. The experiments were performed before the date of expiry of the antivenom.

Neutralisation of different biological properties of Russell's viper venom, such as lethality (5LD₅₀ i.v.), coagulant (10MCD), haemorrhagic (3MHD), necrotic (3MND), defibrinogenating (5MDD) and capillary permeability increasing (100 MCPID) activities was carried out by WHO recommended standard test of neutralising activity [7].

Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, incubated at 37°C for 30 min. and injected into laboratory animals. The end point is

taken as the minimum amount of antivenom required to neutralise 50% of the biological effects of venom.

For determination of neutralisation of lethal activity ED₅₀ (effective neutralising dose) is used. It is the minimum amount of antivenom that will save 50% of the test animals in 24h following the injection.

RESULTS

Biological properties of the Russell's viper venom

Biological properties of the pooled Russell's viper venom are: MHD (21.08ug/rat), MND (19.9ug/rat), MDD (1.2ug/mouse), MCPID (0.0036ug), LD_{50iv} (2.34ug/mouse) and MCD (1.256ug/ml).

Potency assay of the antivenom

Monitoring of the five samples of a batch of the antivenom from 7-15 months following manufacture shows neutralising efficacy of individual antivenom to hemorrhagic, defibrinogenating, coagulant and lethal (except necrotic and capillary permeability increasing) activities remains fairly stable on storage. But within batch variation of

Table 2. Results (mean values) of potency assay of five samples of a batch of antivenom C98011 at 7-9 month and 13-15 month following manufacture

ASV age (mth)	3MHD	3MND	100MCPID	5MDD	5LD _{50iv}	10MCD
	126.5µg/rat	119.4µg/rat	0.724ug	12µg/mouse	23.4µg/mouse	25.12µg/ml
7-9	80 ± 27.38µl	26.5 ± 14.09µl	6 ± 2.23 µl	5 ± 3.06 µl	12 ± 7.58 µl	0.625 ± 0.382µl
13-15	80 ± 27.38	60 ± 22.36*	11 ± 5.47*	5 ± 0	12 ± 4.47	0.750 ± 0.473

Data are means ± SD (n=5)

*P<0.001

mth = Month

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MCPID = Minimum capillary permeability increasing dose

MDD = Minimum defibrinogenating dose

MCD = Minimum coagulant dose

LD_{50iv} = Median lethal dose

neutralising efficacy of the antivenom to different biological activities of the venom is observed (Table1).

Study of the five samples from a batch of the antivenom at 7-9 month and 13-15 month shows a significant reduction in neutralising efficacy of the antivenom to the necrotic (p<0.001) and capillary permeability increasing (p<0.001) activities at 13-15 month compared to that of 7-9 month following manufacture (Table 2).

DISCUSSION

There are few published data on study of stability of liquid antivenom. Enzyme refined antitoxin are more stable than the whole sera preparation and deterioration at temperature between 0 and 5°C is negligible [8]. In general, liquid antivenom stored at different temperatures suffers different rates of activity loss (2-4). Potency of a liquid antivenom is maintained for at least one year when stored at 30°C (3). At 37° C the preparation may loose 10-20% of their activities in one year. According to Christensen [4], liquid antivenom if stored at 37°C for 6 months began to loose its efficacy. Reduction in neutralising potency of the aged antivenom stored at 4°C calls for use of fresh antivenom and practice of rapid

turn over of the stock by the end users. Within batch variation in antivenom potency may be responsible for variable response of antivenom to venom-induced effects seen in Russell's viper bite patients [5].

Variation in potency of antivenom from batch to batch and manufacturer to manufacturer has been reported [9]. Ampoules of one batch of Behringwerke North and West Africa antivenom stored at 22 to 24°C maintained its neutralising potency for over 15 years after date of expiry whereas other batches of the same antivenom deteriorate within the expiry period [9]. Batch-to-batch variation of potency of MPF antivenom in neutralising venom induced effects following Russell's viper bites has been reported [6]. A slight variation in neutralising efficacy of liquid Russell's viper antivenom stored at 18-32°C in a sand pot with daily watering for 6-7 months [10] could be attributed to within batch variation of the antivenom.

It may be concluded that reduction in potency of aged antivenom and within batch variation of the antivenom potency could be attributing factors accounting for failure of antivenom to neutralise some venom-induced effects seen in Russell's viper bite cases.

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Bacteriological aspects of milk and milk products in Yangon during 2000

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A total of 129 samples of milk and milk products were tested for bacterial contamination. These included raw milk (n=15); boiled milk (n=15); condensed milk (n=25); yogurt (n=25); icecream (n=25); and milk curd (n=24). They were collected from street vendors and shops of Pazundaung, Lanmadaw, Tamwe, Thingangyun, Papedan and Dagon townships during January to September, 2000. Total bacterial count ranges from nil to $>10^8$ organisms/ml. Coliforms were present in 109/129 samples (84.50%) and faecal coliforms in 66/129 samples (51.16%). The bacteria isolated were *Escherichia coli* (including *E. coli* O157K+), *Klebsiella* species; *Staphylococcus epidermidis*, *Proteus* species, *Shigella* species including *Sh. dysenteriae* type 1 & *Sh. boydii*; and *Salmonella* species. Generally, raw milk in this study were found to be unsafe for consumption as expected. Other milk products were also found to be unsatisfactory with the most probable number (MPN) of coliforms are much higher than the critical value.

INTRODUCTION

A food borne disease outbreak (FBDO) is defined as an incident in which two or more persons experience a similar illness resulting from the ingestion of a common food. These include a wide range of diseases including diarrhoeal and parasitic diseases. They represent one of the most widespread and overwhelming public health problem in the world especially in developing countries. In Myanmar diarrhoea, dysentery, food poisoning, typhoid and paratyphoid fever are at the top of the list of Disease Under Surveillance (Notifiable Diseases) and they are all food borne diseases [1]. World Health Organization data has shown that each year some 1500 million episodes of diarrhoea occur in children under the age of five; resulting in 3-million deaths [2,3]. Most of the aetiological agents are associated with seasonal changes, environmental and personal hygiene. The

ingestion of food or water is considered to be the principle mode of transmission of enteric pathogens such as *Aeromonas*, *Escherichia coli*, *Salmonella*, *Shigella*, *Mycobacterium tuberculosis*, hepatitis and other enterovirus. With the changes in food production and preparation, knowledge is needed for the recognition of the possible pathogens. There are also the emergences of new and newly recognized types of food borne diseases. Access to good quality, safe and nutritious food is considered a basic right of the people. Consumption of unsafe, contaminated food leads to food-borne diseases which cause considerable morbidity and mortality [4]. The foods most commonly involved in food-borne disease are meat and meat products, poultry, eggs, milk and milk products, sweet meats and rice preparations. From various countries it was claimed that safety measures of food is always required [5] It was reported that food as a source of enteropathogens causing

childhood diarrhoea [6]. Fleming *et al.*, 1985 [7] also reported that pasteurized milk as a vehicle of infection in an outbreak of Listeriosis. Thus, bacteriological examination of milk and milk products need to be determined.

MATERIALS AND METHODS

Sample collection

A total of 129 samples of milk and milk products which included raw milk (n=15); boiled milk (n=15); condensed milk (n=25); yogurt (n=25); icecream (n=25); and milk curd (n=24) were collected from street vendors and shops of Pazundaung, Lanmadaw, Tamwe, Thingangyun, Papedan and Dagon townships during January to September, 2000.

Determination of coliforms and faecal coliforms

The multiple tube method using MacConkey broth purple in double and single strength were used for the determination of total coliforms and faecal coliforms with series of tubes incubated at 37°C and 44.5°C respectively for up to 48 hours. Confirmation was done using Brilliant Green bile broth [8].

Plate Count Method

The sample bottles were shook up and down for 25 times. Dilutions were prepared to obtain 1:10; 1:100, 1:1000, 1: 10,000 & 100,000 with sterile normal saline solution. Mix thoroughly on a mixer and droplets of 0.02 ml of each dilution were introduced onto Nutrient agar plates. The plates were allowed to dry at room temperature and incubated at 37°C overnight according to the method of Miles & Misra, 1938 [9].

Determination of pathogenic bacterial pathogens

Isolation of bacterial pathogens was determined by the method as described in WHO manual, 1980 [10]. Classification was

done as according to Cowan and Steel, 1965 [11]. Gram positive and gram-negative bacteria based on the Family Pseudomonadaceae, Enterobacteriaceae, Micrococcaceae, Lactobacillaceae, Streptococcae and Vibrionaceae were included for identification [12-14]. After centrifugation, the milk samples (approximately 50 ml) placed at 0°C for 15 minutes, they were inoculated on Blood agar (BA), MacConkey agar (MA), Salmonella-Shigella agar (SS), Mannitol Salt agar (MSA), Tomato Juice agar (TJA), and Thiosulphate Citrate Bile Sucrose agar (TCBS) for the isolation of *Streptococcus* spp.; *Salmonella* spp.; *Shigella* spp; *Escherichia coli*, *Lactobacillus* spp. and *Vibrio* spp. Simultaneously, enrichment media of Selenite F and Alkaline Peptone water were used for secondary inoculation.

Biochemical characterization of pathogenic organisms

Biochemical characterization of pathogenic species were determined by using classical biochemical tests according to the standard method. Colonies from the respective nutrient agar were subjected to a short set of biochemical tests. They include Triple Sugar Iron Agar (TSI) for fermentation of glucose and lactose; Lysine Iron Agar (LIA) for lysine decarboxylase; Urea agar for production of urease and Sulphide Indole Motility medium (SIM) for motility and the production of hydrogen sulphide and indole. Oxidase production was done on oxidase test papers.

Serotyping

Serotyping of the isolated strains were done with polyvalent and monovalent antisera of *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholerae* O1 and *Vibrio cholerae* O139.

RESULTS

(1) Presence of coliforms, faecal coliforms from different sources of milk and milk products

As shown in Fig. 1, all raw milk samples

were contaminated with coliforms and faecal coliforms. Coliforms and faecal coliforms identified from various samples were: from boiled milk 73.3% and 53.3%; condensed milk 80% and 28%; yogurt 64% and 36%; ice-cream 100% and 68% and in milk curd 91.7% and 41.7% respectively.

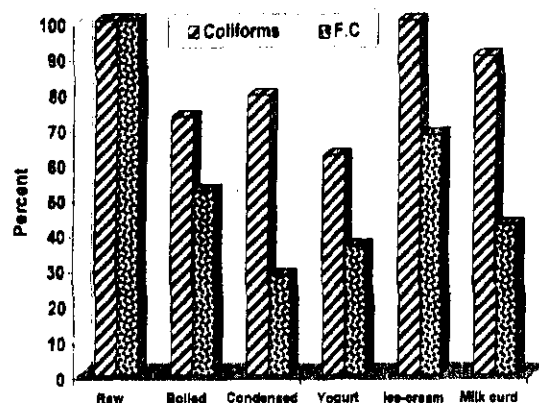


Fig 1. The presence of coliforms and faecal coliforms in milk and milk products

Coliform count from different samples of milk products

As shown in Table 1, the most probable number (MPN/100 ml or gm) of milk and milk products showed that 93.3% of raw milk was contaminated heavily with coliforms with the count of >1800 MPN/100ml. In boiled milk samples only

26.67% were free from coliforms and 60% of them contaminated with the range of 2-300 MPN/100ml. In locally prepared condensed milk 20% were free from coliforms and 80% of them were presence with coliforms within the range of 2-300 MPN/100ml. In yogurt samples, 36% were free from coliforms and 52% had within the range of 2-300 MPN/100ml. In icecream samples all of them were contaminated with coliforms and 80% of them had >1800MPN/100 ml. In milk curd samples, only 8.33% were absent from coliforms and 75% had within the range of 2-300 MPN/100 ml.

Faecal coliform count among different samples of milk

As shown in Table 2, the most probable number (MPN/100 ml or gm) of milk and milk products showed that all of the raw milk samples were contaminated with faecal coliforms and 46.67% of them were contaminated heavily with the count of >1800 MPN/100ml. In boiled milk samples only 46.67% were free from faecal coliforms and 40% of them were contaminated with the range of 2-300 MPN/100ml. In locally prepared condensed milk 72% were free from faecal coliforms and 28% of them were presence with

Table 1. Presence of coliforms from different samples of milk and milk products

Milk Samples	No. Tested	Absent 0 = <2	Present 2- >1800	Coliform count MPN/100ml			
				2-300	301-600	901-1200	>1800
Raw	15	nil (0)	15 (100.00)	1 (6.67)	nil	nil	14 (93.33)
Boiled	15	4 (26.67)	11 (73.33)	9 (60.00)	nil	nil	2 (13.33)
Condensed	25	5 (20.00)	20 (80.00)	20 (80.00)	nil	nil	nil
Yogurt	25	9 (36.00)	16 (64.00)	13 (52.00)	1 (4.00)	nil	2 (8.00)
Icecream	25	nil	25 (100.00)	1 (4.00)	3 (12.00)	1 (4.00)	20 (80.00)
Milk curd	24	2 (8.33)	22 (91.67)	18 (75.00)	1 (4.17)	nil	3 (12.50)
Total	129	20	109	62	5	1	41

Figures in parenthesis denote percentages

MPN = Most Probable Number

Table 2. Presence of Faecal coliforms from milk and milk products

Milk Samples	Absent 0 = <2	Present 2- >1800	Faecal coliform count MPN/100ml				
			2-300	301-600	901-1200	1501-1800	>1800
Raw n=15	nil (0)	15 (100.00)	4 (26.67)	nil	1 (6.67)	3 (20.00)	7 (46.67)
Boiled n=15	7 (46.67)	8 (53.33)	6 (40.00)	1 (6.67)	nil	nil	1 (6.67)
Condensed n=25	18 (72.00)	7 (28.00)	7 (28.00)	nil	nil	nil	nil
Yogurt n=25	16 (64.00)	9 (36.00)	0 (36.00)	nil	nil	nil	nil
Icecream n=25	8 (32.00)	17 (68.00)	12 (48.00)	nil	1 (4.00)	1 (4.00)	3 (12.00)
Milk curd n=24	14 (58.33)	10 (41.67)	10 (41.67)	nil	nil	nil	nil
Total=129	63	66	48	1	2	4	11

Figures in parenthesis denote percentages

MPN= Most Probable Number

Table 3. Total Bacterial Count obtained from milk and milk products

Bacterial count/ml	Raw n=15	Boiled n=15	Condensed n=25	Yogurt n=25	Icecream n=25	Milk curd n=24
nil	0	4 (26.67)	9 (36.00)	6 (24.00)	2 (8.00)	7 (29.17)
0.5-10 ³	1 (6.67)	2 (13.33)	5 (20.00)	3 (12.00)	1 (6.67)	6 (25.00)
10 ⁴	nil	nil	3 (12.00)	8 (32.00)	4 (16.00)	2 (8.00)
10 ⁵	nil	3 (20.00)	2 (13.33)	5 (20.00)	3 (20.00)	nil
10 ⁶	6 (40.00)	1 (6.67)	3 (12.00)	1 (6.67)	4 (16.00)	1 (6.67)
10 ⁷	6 (40.00)	3 (20.00)	1 (4.00)	1 (4.00)	2 (8.00)	4 (16.67)
10 ⁸	1 (6.67)	nil	1 (6.67)	nil	1 (6.67)	nil
UC	1 (6.67)	nil	nil	nil	4 (16.00)	nil
NT	nil	2 (13.33)	1 (6.67)	1 (6.67)	4 (16.00)	4 (16.00)

NT = Not tested

Figures in parenthesis denote percentages

coliforms within the range of 2-300 MPN/100ml.

In yogurt samples, 64% were free from faecal coliforms and 36% had within the range of 2-300 MPN/100ml. In ice-cream samples all of them were contaminated with faecal coliforms and 12% of them had >1800MPN/100 ml. In milk curd samples,

only 58.33% were absent from coliforms and 41.67% had within the range of 2-300 MPN/100 ml.

Total bacterial count of milk and milk products

The total bacterial count from milk and milk

products was shown in Table 3 and the count varies from 0.5×10^3 to uncountable numbers.

Bacterial species isolated from milk and milk products

Isolation of different species of bacteria were shown in Table 4. *Pseudomonas* species, *Vibrio cholerae* O1 and O139 were not isolated in these samples.

DISCUSSION

Food poisoning is a major public health concern worldwide. Mass food production, catering and wide distribution of food would undoubtedly increase the incidence of food poisoning especially if there is improper or unhygienic food handling or preparation. Awareness of emerging infectious disease is also important [14]. The food itself provides

Table 4. Bacterial species isolated from milk and milk products

Organisms	Raw n=15	Boiled n=15	Cond- ensed n=25	Yogurt n=25	Ice cream n=25	Milk curd n=24
<i>Escherichia coli</i>	2 (13.33)	1 (6.67)	nil	3 (2.0)	2 (8.0)	nil
<i>Klebsiella</i> spp.	nil	5 (33.33)	3 (12.0)	3 (2.0)	4 (16.0)	3 (12.5)
<i>Proteus</i> spp.	nil	2 (13.33)	3 (12.0)	nil	3 (12.0)	nil
<i>Salmonella</i> spp.	nil	nil	1 (4.0)	nil	2 (8.0)	1 (4.17)
<i>S. epidermidis</i>	1 (6.67)	nil	1 (4.0)	nil	4 (16.0)	1 (4.17)
<i>E. coli</i> + <i>Klebsiella</i> sp.	1 (6.67)	1 (6.67)	nil	1 (4.0)	1 (4.0)	nil
<i>E. coli</i> + <i>Proteus</i> sp.	2 (13.33)	1 (6.67)	nil	1 (4.0)	nil	nil
<i>E. coli</i> + <i>S. epidermidis</i>	4 (26.67)	nil	nil	nil	4 (16.0)	3 (12.50)
<i>Klebsiella</i> + <i>Proteus</i>	1 (6.67)	1 (1.67)	nil	nil	nil	nil
<i>Klebsiella</i> + <i>S. epidermidis</i>	nil	nil	4 (16.0)	nil	2 (8.0)	1 (4.00)
<i>Salmonella</i> + <i>S. epi.</i>	nil	nil	1 (4.00)	nil	1 (4.00)	nil
<i>Salmonella</i> + <i>Klebsiella</i>	nil	nil	nil	nil	nil	3 (12.50)
<i>Kleb.</i> + <i>Proteus</i> + <i>S. epi.</i>	nil	1 (1.67)	nil	nil	nil	nil
<i>E. coli</i> + <i>Kleb.</i> + <i>S. epi.</i>	nil	nil	nil	nil	1 (4.00)	nil
<i>E. coli</i> + <i>S. epi</i> + <i>Sh. boy.</i>	1 (6.67)	nil	nil	nil	nil	nil
<i>Proteus</i> + <i>Kleb.</i> + <i>Sh. dys.</i>	1 (6.67)	nil	nil	nil	nil	nil
<i>E. coli</i> + <i>Klebsiella</i> + <i>S. epidermidis</i> + <i>Proteus</i>	2 (13.33)	nil	nil	nil	nil	nil
Not isolated	nil	3 (20.0)	12 (48.0)	17 (68.0)	1 (4.0)	13 (54.17)

Figures in parenthesis denote percentages

sufficient nutrients and with adequate moisture, warmth and time lapse between preparation and consumption of food, multiplication of the pathogen will inevitably take place.

Many of the coliforms are inhabitants of the human and animal intestine. Coliform can survive and grow in an environment associated with food processing in which other enteric pathogens may not survive. Coliforms rather than *Escherichia coli* are good indicators of unsatisfactory processing or sanitation and the presence of large numbers of coliforms in processed foods indicate that the opportunity for proliferation has occurred. Coliforms can persist and grow in improperly cleaned equipments and utensils. They can establish themselves as part of the resident flora of food processing establishments where they are difficult to eliminate. Coliforms may also enter the food products after processing from the hands or garments of food-handlers, or from dirty utensils or from unsafe water used.

Coliforms do not necessarily indicate contamination from faecal source. However, it does indicate inadequate processing or post processing contamination, most probably from workers, or from dirty equipments or from the raw food before processing. The presence of large number of coliforms does not necessarily indicate an immediate health hazard, but it indicates lack of good sanitary practice. Presence of faecal coliforms indicate faecal-oral contamination. *Escherichia coli* does not usually persist for a long time in the environment other than the intestine. Thus, the presence of *Esch. coli* in foods generally indicates recent pollution of faecal origin. Recent pollution of faecal origin may be contamination directly by faeces or indirectly by faecally contaminated materials [14].

One aspect of food-borne infections can be attributed especially to the use of unsafe

water in preparation of foods and washing the utensils. Other factors that are known to contribute to the risk of a foodborne disease include contamination from infected or colonized food handlers, inadequate boiling temperatures, improper holding temperatures, contaminated equipments and utensils. Thus, food preparation and handling process play a key role in causing food poisoning outbreaks.

In Myanmar, traditional milk product is used in the preparation of sweets. It is rich in nutrients and has high water activity which is conducive for the growth of bacteria. Most of them were contaminated with pathogenic organisms such as *Staphylococcus* and *Escherichia coli*. Though the microbiological quality of milk was satisfactory at the time of production, it deteriorated by the time it is displayed for sale at the market place. Various brands of Ice-cream tested for bacteriological examination revealed that almost all were contaminated with coliforms and only 32% of the samples were free from faecal coliforms. Meanwhile some brands of yogurts were also contaminated with coliforms and faecal coliforms.

The general requirements for prevention of microbial food poisoning from could be applied to *Bacillus cereus* and *Esch. coli* to be used in well establish hygienic principles in the manufacturing, preparation, storage and serving food, training of food service personnel; health education, licensing of food service establishments depending on recognized qualifications in food hygiene for their managers and supervisors and compliance with the National Food Regulation.

For the prevention of the multiplication of microorganisms in cooked, fast foods are: to prepare the food immediately before serving (or) to be cooled after cooking and stored at appropriate temperature (chilling) that prevent bacterial growth (or) to be held in warm state, above 60°C of temperature.

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Venom ophthalmia following spitting of venom into the eyes by spitting cobra (*Naja mandalayensis*) in Myanmar

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A retrospective study of eight venom ophthalmia cases including five reptile keepers following spitting of venom into the eyes by spitting cobra (*Naja mandalayensis*) in Myanmar was reported. Severe burning pain, profuse watering of the eye, conjunctivitis, itchiness and palpebral oedema that lasted overnight in the mild and 3 days in the severe cases were observed. No visual impairment was observed except in one who presented with temporary loss of vision for 3 days. Immediate irrigation of eye with a large volume of water is recommended as a first aid measure. The study highlighted the need for educating reptile handlers about taking safety precautions at work and first aid measures. Moreover, these snakes should not be kept in an open snake pit and carries risk to public.

INTRODUCTION

Snakebite is a common problem in Myanmar. Russell's viper (*Daboia russelii siamensis*) bite constitutes about 90%, Cobra (*Naja kaouthia*) 5.2% and green pit viper (*Trimeresurus erythrurus*) 3.8% [1]. There are occasional reports of Chinese krait [1] and seasnake [2] bites. However, venom ophthalmia caused by spitting cobra (*Naja mandalayensis*) (Figure 1) has not been reported before in Myanmar. In this communication a retrospective study of clinical features and outcome of eight cases of venom ophthalmia were reported.

Patients

Nine-year old boy from Saeto village, Mahlaing Township, Mandalay Division, fell from a height of one metre from a tree following spitting of venom to his eyes on 15 November 1992. Local examination at the station hospital revealed that his eyes were swollen and his face covered with specks of venom. He complained of

itchiness and burning pain in the eyes. Irrigation of the eyes with a large volume of water followed by local instillation of antibiotic and padding were carried out. Except for conjunctivitis, he suffered no visual defect. The facility for examining eyes under slit lamp or fluorescein was not available at the local station hospital.

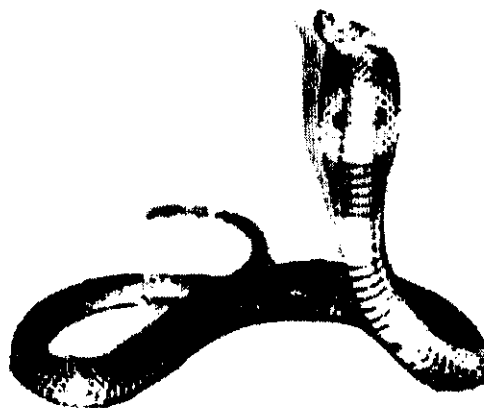


Figure 1. A spitting cobra (*Naja mandalayensis*) caught from Kyauksae, Mandalay Division.

Another accident occurred to a 17 yr old girl from Myakanthar village, Singu Township, Mandalay Division on 17 July 1997. She noticed a cloud of fume rushed toward her eyes and then she could not see light or objects any more. After irrigation of the eyes with a bowl of water, she had painful watery swollen red eyes that lasted overnight. Local examination of the eyes at the Yaenanthar district hospital 14h after the incidence revealed that both eyes were normal except pupils were not reacting to light. She could neither count fingers nor see light. No features of systemic neurotoxic envenoming were detected. Light perception returned on the second and full vision on the third day after the admission. Steroid eye drops were instilled, an eye pad applied and systemic steroid was given.

Six employees of the Zoological gardens and the snake farm of MPF suffered from venom ophthalmia while at work. A 27 yr old zoo employee was spat by a spitting cobra at a distance of one metre from a metre deep snake pit in Mandalay Zoo. She had severe burning swollen itchy eyes that persisted for 3 days. No visual defect was reported.

Five reptile handlers were spat by spitting cobras while counting snakes. All suffered from swollen itchy burning pain in the eyes that lasted overnight. Eyes were rinsed with a large volume of water. No visual impairment was reported. Local instillation of antibiotic was carried out only. The incident occurred in the respective places following deposition of 100 spitting cobras to Mandalay and 50 to Yangon Zoos and 600 to the snake farm of MPF.

DISCUSSION

Venom ophthalmia following spitting of venom into the eyes has not been reported in Myanmar before. This is the first report of 8 venom ophthalmia cases following spitting of spitting cobra (*Naja mandalayensis*) in

Myanmar. It highlighted that it is an occupational hazard for snake handlers. Of the nine species of Asian Cobra, *N. sumatrana*, *N. sputatrix*, *N. siamensis*, *N. philippinensis* and *N. atra* have been known to spit venom frequently [3-4]. Public including the keepers have not encountered spitting cobra before. Lack of information on venomous snakes of Myanmar is responsible for the accidents. In this case mistaken spitting cobras were brought to the Zoos for display and to the snake farm as cobras from venom extraction. Although the victims are from different localities, the snakes originated in mid-land, Mandalay Division where the very first reported case came from. The species is named *Naja mandalayensis* by JB Slowinski [5]. According to Wuster and Thorpe [6], *N. siamensis* is found probably in parts of eastern Myanmar.

Pain, conjunctivitis and palpebral oedema have resulted from spitting by *N. sputatrix* in Java [3], *N. sumatrana* in west Malaysia and *N. siamensis* in Thailand [7]. A similar observation was made in our study, however severe burning pain and excessive lacrimation accompanied by itchiness is marked in ours. Five cases of spitting of venom into the human eyes by *N. atra* in Guangxi were reported [8].

Absorption of venom into anterior chamber of the eye resulted in hypopyon and anterior uveitis had been reported [9]. Accidental entry of *N. kaouthia* venom into the eyes of a reptile handler while attempting to break fang, resulted in intolerable, excruciating painful conjunctivitis and palpable oedema (personal communication). A transient VII nerve palsy on the affected side of accidental entry of *N. naja* venom into one eye, probably due to neuritis following tracking of venom absorbed through the conjunctiva was observed (Warrell, D.A., personal communication). Probably temporary loss of vision in our case is result of local absorption of venom through conjunctiva leading to retinitis and optic neuritis.

The study highlighted that reptile handlers should be educated about taking safety precautions and first aid measures. It is an occupational hazard of snake handlers who lack information on spitting cobra. Irrigation of eye with a large volume of water is recommended as a first-aid measure. Since spitting cobra is capable of spitting venom to a distance of 2-3 metres, it should be kept in a glass compartment for display otherwise public are at risk as in one of our victims.

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45 days mechanical ventilation in a Chinese krait (*Bungarus multicinctus*) bite

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The clinical course of a severe envenomed Chinese krait (*Bungarus multicinctus*) bite that required mechanical ventilatory support for 45 days was described. Severe muscle pain in the chest and abdomen developed ½h after the bite and heavy upper eyelids 1h after the bite. He became drowsy with peripheral cyanosis 11h later and required mechanical ventilatory support for 45 days in intensive care unit. Twenty-four hours after the bite, pupils were dilated, and all limbs became flaccid but he remained conscious through out the course. Spontaneous limb movement began 60h after the bite, eye movement on day 7, movement of all limbs on day 44 and spontaneous respiration on day 45. *Bungarus fasciatus* antivenom from Thai Red Cross and Haffkine given at 34 and 61h after the bite respectively failed to revert the neurotoxicity and Chinese krait (*Bungarus multicinctus*) antivenom given on day 12 produced little dramatic effects on recovery.

INTRODUCTION

Snakebite is common in Myanmar. Russell's viper (*Daboia russelii siamensis*) bite is an occupational hazard for farmers and constitutes about 60% (8.2% fatality) of the total bites. Bite from cobra (*Naja kaouthia*) (6% fatality 8%), green pit vipers (5%), unknown bites (29% fatality 3%) are also reported [1]. Chinese krait (*B. multicinctus*) bite is rarely reported probably some cases may have mistakenly reported as cobra bite. However, its bite usually occurs at night while asleep on ground. There were reports of Chinese krait bites in Taiwan [2], Southern China [3] and Hongkong [4]. Following enquiry, we came across 8 cases of Chinese krait bites [5]. Recently, we came across two more cases; one with severe neurotoxic envenoming that deserves reporting.

Patient

This severe bite took place at Yangon Zoological garden at 9 am on 18 June 1997

following donation of a Chinese krait to the Zoo. 20 yr old keeper was bitten on right forearm following grasping of the wound present on dorsum of middle third of the snake (Figure 1). He continued his routine work until ½h later, he felt giddy and developed aching pain in the muscle of the chest extending to involve muscle of the throat, face and lips accompanied by nausea and vomiting. One hr after the bite, he could speak but had difficulty in opening eyes and lifting up upper eyelids.



Fig. Picture of a Chinese krait (*Bungarus multicinctus*) responsible for the bite. Arrows indicate the sites of wound

He was admitted to Intensive care unit of Yangon General Hospital 1.50h after the bite. Locally there was slight swelling and pain. Fang marks could not be identified. He had partial ptosis and severe aching pain in the chest and abdomen. Blood was clottable on admission. At 7.50h he developed muscular weakness and became drowsy, but SaO_2 was 99% with FiO_2 50%. Twelve hours after the bite, SaO_2 suddenly fell to 80% with FiO_2 50%, BP 140/100 mmHg, HR 120/min and GCS 3/15. The patient was intubated with cuffed endo-tracheal tube without any pharmacological aid and mechanical ventilatory support was instituted with controlled mechanical ventilatory (CMV) mode.

Twenty-four hours after the bite, the patient was awake though all limbs were flaccid. BP was 140/100mmHg and HR 120/min. Thirty-four hours after the bite, 20ml of banded krait (*B. fasciatus*) antivenom (Thai Red Cross) were infused, followed by hourly infusion of 3 doses of 10 ml of the antivenom. No improvement in neurological symptoms was observed. Sixty-one hours after the bite, 10ml banded krait (*B. fasciatus*) antivenom (Indian Haffkine Institute) was given i.v. hourly for 5 doses. His blood pressure measures 155/70 mmHg on day 3. Eye movement returned on day 7 but light reaction was sluggish. Could retract upper eyelids. Tracheostomy was carried out. On day 8, he could protrude tongue and lift eyes brow. By day 11 ptosis was improved mid way and his BP measures 150/90 mmHg. Intravenous injection of 5 ml (800 iu) *B. multicinctus* antivenom (China) was carried out at 8pm on day 12. Another infusion of 5 ml *B. multicinctus* antivenom (China) in 500 ml of normal saline was given at 8am on day 14. Another infusion of 5 ml *B. multicinctus* antivenom in 100 ml dextrose saline was repeated at 12 noon on day 16. Ventilator was switched off and on 1-2h only on day 42. He could move all 4 limbs on day 44. Ventilator was switched off on day 45. Breathing through tracheostomy tube was

continued. Tracheostomy closure was carried out on day 85. Rehabilitation was done and he could walk out on transfer-back on day 112.

DISCUSSION

The clinical course of a Chinese krait (*B. multicinctus*) bite that needed 45 days mechanical ventilatory support was described. It is the longest duration of mechanical ventilatory support given to neurotoxic snakebite cases in our intensive care unit.

Early onset of neurotoxic symptoms is due to rapid absorption of the venom aided by lack of immobilization of the bitten part and first aid. Clinical features of envenoming were similar to those observed in other *B. multicinctus* bites [2-4]. In our case the patient had a natural recovery with ventilatory support rather than due to specific antivenom given 12 d after the bite. Even specific antivenom could not relied upon to reverse neurotoxicity has been reported [6-7]. Response to anticholinesterase and neostigmine in reversing neurotoxicity was not observed in the present case as well as in our previous case [6]. The indication for mechanical ventilatory support was purely ventilatory failure from muscle weakness.

The study also highlighted that natural recovery could be expected with proper ventilatory support even if the specific antivenom is not available. Survival of one severe envenomed patient of *B. multicinctus* bite following 30 days of mechanical ventilation has been reported earlier [8]. The same principle applied to other neurotoxic envenoming cases. It also showed that delay administration of specific antivenom plays little role in reversing neurotoxicity. No cardiac, renal, hepatic or haematological toxicity is documented. Early referral to intensive care unit is vital in neurotoxic envenoming snakebite cases.

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SHORT REPORT

Social aspects of leprosy patients in Hmawbi Township

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Leprosy is no longer the disease of lost hopes for the patient, the family and the community. The disease is curable today with multidrug therapy. But the disease is still challenging to us due to the problems associated with disabilities. Though leprosy is a curable disease, social impact against the disease were observed elsewhere. In India, families with deformed patients were found with problems ten times higher than those with no deformities [1]. So also high percentage of family and community of Myanmar community were shameful to have patients in family and community of Myanmar or disfigured patients [2]. Thus social stigma against leprosy is important for patients, their families and community, and this study attempted to explore the social aspects of leprosy patients of Hmawbi Township.

A cross-sectional study was done in Hmawbi Township in 1996 for two months. After excluding the untraceable, monks and patients below the age of 16 years, the study involved 101 patients with the response rate of 95.3%. They were interviewed with pretested structured questionnaire by the research team. The questionnaire was based on the finding of focus group discussion (FGD) among patients with disabilities and without disabilities. FGD was conducted in Hlegu Township.

Not all the patients had encountered with social problems. Patients with disabilities had faced with more problems than those with no deformities. With regard to education, only one patient said that his disease affected his children's education. While other patients had no problems with their jobs, five patients had changed their jobs due to the disease. Only two patients with disabilities faced with marital problems, such as divorce, and four patients faced negative attitudes of their in-laws. Leprosy-oriented divorce was found as one of the causes of not having the child in one patient.

Although the socio-economic problems were found to be not the major obstacle for leprosy patients, some social stigma against the disease was observed among them. Concealment about the disease was found while attending the school (9%) and five out of 11 patients did not tell their spouses. Regarding their children's marriage, 22 patients said they would not allow their children to marry to those from families with disease. With respect to social functions, while six patients said that they were not invited, 13 patients did not attend even they were invited. Most patients were accepted either by their families or community, however, eight patients felt of being treated as outcasts by society, two

patients thought that death would be the solution for solving problems and six patients felt of depression.

Unlike the other social studies, this study showed that only a few patients were faced with problems relating to education, occupation, marriage and social. In spite of intense social stigma, it was noted that social acceptance was very much satisfactory. Though the family members were aware of the patient with leprosy, there were no changes of social dealing to those persons affected by leprosy. It could be the nature of rural culture. The findings also revealed that, health education activities related to leprosy are necessary for the patients and families as some cured patients are still thinking that they have not been totally cured yet.

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SHORT REPORT

Batch to batch variations in Russell's viper (*Daboia russelii siamensis*) venom

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Myanmar Pharmaceutical factory (MPF) manufactures two monospecific (*Daboia russelii siamensis* and *Naja kaouthia*) antivenoms that are used for treating specific snakebite cases through out the country. Venoms milked from locally available Russell's vipers were pooled and used as immunogen for raising antivenom. These were not characterised. However, study of three yearly-pooled Russell's viper venom (1981, 1982 and 1983) collected from Kungyankone Township, Yangon Division shows variation in coagulase activity and L amino acid oxidase activity between pools [1]. Variation in some enzymes and biochemical activity of venom was also observed in samples obtained from three fortnightly milked venoms from 4 Russell's viper has been reported [1]. However, batch-to-batch variation in potency of antivenom [2] also suggested that variation in properties of immunising venoms may account for this. The study attempts to answer the assumption.

A total of 9 batches (6 primary=first time milking and 3 secondary=second time milking) of venom milked from a pooled of 150-350 Russell's vipers (*Daboia russelii siamensis*) kept in the snake farm of MPF were desiccated with calcium chloride. Milking is carried out between 28 June and 12 December 1990. The interval between different primary milking ranges from 4 to 19 days following captivity and secondary

ranges from 11 to 25 days after the first. Biological properties such as lethality, haemorrhagic, coagulant, necrotic, defibrinogenating and capillary permeability increasing activities of the venom, were tested according to WHO recommended techniques [3]. Biochemical activity like arginine esterase activity was measured by using substrate TAME [4], phospholipase activity using substrate phosphatidyl choline [5] and L amino acid oxidase activity using L leucine as substrate [6] and pyruvate formed was measured following the development of colour with dinitro phenyl hydrazine substrate. Enzyme activity is expressed in units/mg/min.

Results of biological and biochemical properties of different batches of venom are shown in the table.

Variation in biological and biochemical activities of different batches of venom is observed. There is an inter batch variation in biochemical: phospholipase and arginine esterase activities and protein content ($p < 0.001$), biological: LD₅₀ ($P < 0.01$), haemorrhagic and defibrinogenating activities ($p < 0.05$) in the primary milking and in biochemical: phospholipase and arginine esterase activities and biological: haemorrhagic (MHD) ($p < 0.001$), necrotic (MND) ($p < 0.01$) and LD₅₀ and defibrinogenating (MDD) activity ($p < 0.05$) in the secondary. No significant variation in

coagulant (MCD) activity is observed in both milkings. Differences in biochemical:

Our findings support the recommendation made by the WHO that venom used for

Table. Variation in biological and biochemical activities of different batches Russell's viper venoms

Batch No./milking*	LD ₅₀ µg/18-20g mouse**	MHD µg/rat	MND µg/rat	MDD µg/mouse	MCD µg	MCPID µg/ml	Phospho-lipase activity*** unit/mg/ min	Arginine esterase activity*** unit/mg/ min	L-Amino acid oxidase activity *** unit/mg/ min	Total protein µg/ml
0190/P	3.06±3.9	50.1	31.6	4	0.1585	0.007	1260 ± 0.7	280 ± 1.3	170 ± 0.9	880
0390/P	5.4 ± 2	51.8	31.6	5	0.1072	0.089	1310 ± 1.0	295 ± 0.6	151 ± 0.2	795
0690/P	7.14 ± 1.7	46.8	56.2	7	0.0178	>1mg/ml	1130 ± 0.2	360 ± 1.1	188 ± 0.3	970
1290/P	13.36 ± 2.4	60.3	51.3	10	0.1738	>1 mg/ml	1320 ± 1.7	311 ± 0.5	280 ± 0.9	965
1590/P	4.8 ± 3.1	48.4	31.6	4	0.3162	0.158	1260 ± 1.1	366 ± 0.3	253 ± 0.7	870
1690/P	14.15 ± 3.3	63.1	51.2	10	0.0398	>1mg/ml	1420 ± 2	342 ± 0.4	168 ± 0.9	957
2190/S	6.04 ± 3.5	79.4	72.4	3	0.1995	0.1514	1290 ± 0.6	151 ± 0.4	188 ± 0.2	890
2790/S	1.87 ± 2.4	46.2	39.8	2	0.1122	0.354	1132 ± 3	300 ± 0.6	190 ± 0.5	900
3490/S	8.06 ± 2.6	90.2	63.1	5	0.0316	0.5012	1632 ± 0.6	291 ± 1.3	196 ± 0.1	900

* P= Primary milking and S= Secondary milking

Data are means of duplicate determination

** 95% fiducial limits

*** means ± SD (n=4)

MCD = minimum coagulant dose

MHD = minimum haemorrhagic dose

MND = minimum necrotic dose

MDD = minimum defibrinogenating dose

MCPID = minimum capillary permeability increasing dose

LD₅₀ = lethality

Total protein was determined using a concentration of 1 mg dry weight venom per ml of distilled water.

phospholipase, arginine esterase and L amino acid oxidase activities and protein content and biological: haemorrhagic, necrotic and LD₅₀ activities in between the primary and the secondary milkings ($p < 0.001$) are observed. Intravenous LD₅₀ correlates with defibrinogenating and capillary permeability increasing (MCPID) activities and MDD with MCPID of the venom. MCPID activity of the venom is not detected in 3 batches of venom (0690, 1290 and 1690) and these have weak biological activities (except coagulant activity in one) (Table). Variation in electrophoretic pattern of different batches of venom is also observed.

It has been shown that variation in venom properties may occur in different milkings of individual snake [1] and from yearly pools [1]. A similar variation in properties of venoms from lots of *Echis carinatus* and *Echis coloratus* had been reported [7].

raising antivenom should be characterised in order to raise potent antivenom and widely pooled venom should be used for this purpose [8]. Recent observation of batch to batch variations in performance of antivenom [2] and variable performance of a batch of antivenom with different Russell's viper (*D.r.siamensis*) venoms [9] suggested that immunogen used for raising antivenom should be widely pooled and characterised.

Weak antivenom will be produced if it is raised with a batch containing weak immunogens like (0690, 1290, 1650) which may account for failure of correction of some venom-induced defects seen in some antivenom treated Russell's viper bite cases in Taungdwingyi [10] and Danuphyu [11]. Kornalik and Taborska [12] suggested that venom samples with the highest biological activity should be used for immunisation in order to produce more effective antivenom. Since a spectrum of weak to potent

biological activities is present in different batches of venom, it is best to pool them and characterised before use for immunisation.

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MYANMAR HEALTH SCIENCES RESEARCH JOURNAL
INDEX TO VOLUME 14 (2002)

AUTHOR INDEX

A

Aye Aye Myint - 27,
31,42, 50
Aye Than - 1,7,17

C

Cho Cho Oo - 22

H

Htein Lin - 22

K

Khin Aung Cho - 50
Khin May Ni - 1
Khin Myint Wai - 48
Khin Pyone Kyi - 27
Kyaw Myint - 48
Kyi Kyi Mar - 12
Kyi Kyi Myint - 7
Kyi Kyi Thin - 35
Kyi May Htwe -
27,31,50

L

Le Le Win - 48

M

Mar Mar Nyein - 35
May Aye Than - 7,17
Mon Mon - 12
Mu Mu Sein Myint -
1,7,17
Mya Pwint Aye - 48
Myint Thuzar Thant - 17
Myo Tint Tun - 42

P

Pearson, Alan - 22

S

San San Aye - 12
San San Myint - 7
Saw Saw - 12
Soe Naing - 12

T

Than Than Htay - 1
Thandar Myint - 17
That Htut - 42
Thazin Myint - 7
Theingie - 50
Thet Thet Khine - 35
Tin Myint - 1, 45
Tin Nu Swe - 7, 17
Tun Pe - 27, 31, 42, 45,50

W

Win Myint - 1
Win Win Mar - 12

Z

Zin Mar Aye - 12

SUBJECT INDEX

A

Analgesics - 7
Antivenins - 27, 31, 45, 50

B

Bacteriological Techniques - 35
Bungarus - 45

C

Cathartics - 17
Cobra Venoms - 42

E

Endophthalmitis - 42

F

Family Practice - 12

G

General Practice see Family Practice

H

Hypoglycemia - 1

K

Knowledge, Attitudes, Practice - 22
Krait see Bungarus

L

Leprosy - 48

M

Malaria - 22
Milk - 35

N

Naja Venom, see Cobra Venoms
Neutralization Tests - 27

O

Ophthalmia see Endophthalmitis

P

Pain Measurement - 7
Plant Extracts - 1
Plant leaves - 17
Plants, Medicinal - 1, 7, 17
Purgatives see Cathartics

R

Respiration, Artificial - 45
Russell's Viper - 27, 31, 50

S

Snake Bites - 27, 50
Snake Venoms - 27, 31
Social Desirability - 48

T

Tuberculosis - 12

V

Ventilation, Mechanical see Respiration,
Artificial

