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Pharmacokinetics of mefloquine in uncomplicated Plasmodium falciparum malaria patients in Myanmar

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With the aim to study the pharmacokinetic basis underlying the therapeutic outcome of mefloquine in malaria patients, a single oral dose of 1000 mg mefloquine hydrochloride (Mepha) was administered to 24 patients with acute uncomplicated falciparum malaria and 10 healthy volunteers and was followed-up till day 42. The drug serum levels at various time intervals were analysed by a Waters HPLC and the pharmacokinetic profile studied. The study shows that although Myanmar malaria patients had a slower rate of absorption ($t_{1/2ab} = 6.38 \pm 0.60$ hour; $t_{max} = 1.31 \pm 0.12$ days), the peak serum level reached was much higher ($C_{max} = 2.24 \pm 0.05$ ug/ml) than those of the westerners.

INTRODUCTION

The antimalarial drug, mefloquine, which has been widely used in many countries is now becoming increasingly popular in Myanmar. Extensive clinical and field trials have shown it to be effective in the treatment and prophylaxis of malaria caused by Plasmodium falciparum including strains resistant to chloroquine and sulfadoxine-pyrimethamine [1-4]. Although pharmacokinetics of mefloquine has been studied by various authors as early as 1979 [6-8], no such study has been done in Myanmar.

This study was conducted with the object:-

- (1) To compare the pharmacokinetics of mefloquine between patients with uncomplicated P.f. malaria and healthy volunteers.
- (2) To find out the significance and association between pharmacokinetic and pharmacodynamic parameters, so as to understand

the basis underlying the therapeutic outcome in Myanmar patients.

MATERIALS AND METHODS

Drugs

Test drug - Mefloquine hydrochloride tablets, 250 mg (Mepha)
Standard - Mefloquine hydrochloride authentic powder (Mepha)
Internal Standard - 2,8 Bistrifluoromethyl-quinoline-4-carboxylic acid, all supplied by Mepha Ltd., Aesh-Basle, Switzerland.

Subject selection

A total of 24 patients (14 from No. 2, Military Hospital and 10 from Tharyarwaddy Civil Hospital), admitted for signs and symptoms of malaria and confirmed by the presence of asexual P.f. malaria parasites in

blood smear were selected according to the following criteria:

1. Both sexes
2. Age 19-60 years
3. Body weight of at least 40 kg
4. No clinically detectable complications, organic disease of dysfunction.
5. Significant initial parasite count of greater than 1,000 P.f. per microliter.
6. Have not been taking any anti-malarial therapy within the past 2 weeks.

Ten non-infected healthy volunteers of comparable age and body weight were also selected. All participants gave a written informed consent.

Baseline clinical and laboratory assessment

Patients were thoroughly screened on admission and were monitored daily throughout the study period. A standard history, physical examination and laboratory investigations including malaria parasite count, PCV, CP, LFT, serum electrolytes, urine for RE and identification of currently used antimalarial (eg. quinine, chloroquine, sulfonamide etc.) in the urine were done.

Trial procedure

All eligible patients were admitted to the hospital for the minimum of 7 days and were followed weekly until day 42. Mefloquine 1000 mg (4 tablets) single oral dose was given immediately after a control blood and urine sample. Blood sample was continued at 1,2,4,6,12,48 hours and on days 3,7,14,28 and 42 after drug administration. Smoking and all other medications were prohibited except for those required for emergency situations. The serum was separated immediately and stored at -20°C and was analysed within 2 weeks using the HPLC assay method

of Grindel [9].

Data analysis

The drug concentrations were then plotted on a semi-log graph paper and the pharmacokinetic parameters calculated accordingly. Comparison of pharmacokinetic parameters between different parameters and clinical and laboratory findings were done by linear regressive analysis.

RESULTS

A total of 24 uncomplicated *P. falciparum* malaria patients and 10 healthy volunteers took part in the study and their clinical and laboratory data are shown in Table 1. All patients had history of having signs and symptoms of acute malaria and met all the required criteria for entry into the study. The study groups were comparable for age and weight and more than half of the patients gave history of previous attack of malaria. Haemoglobin, initial parasite count, parasite clearance time and fever clearance time were also comparable with the exception of 1 patient from group A who showed R1 resistance, the parasitaemia which initially cleared at 96 hours later reappeared on day 28 despite having a serum mefloquine concentration of 0.41 ug/ml.

Figure 1 shows the mean serum concentration-time profile of mefloquine in the 3 groups studied. The serum concentration-time profile of mefloquine, up to a sampling time of 28 days was found best-fitted to a 2 compartment open model with the elimination of the blood level curve showing a non-linear biexponential decay. This includes a rapid phase having a half-life of 19-36 hours and an extremely slow terminal phase having a half-life of 2-3 weeks. In contrast, the absorption phase was

Table 1. Mean clinical and laboratory data of healthy volunteers and malaria patients

Clinical and laboratory data	Healthy volunteers	Patients with <i>P. falciparum</i> malaria	
		Group A (No. 2 MH)	Group B (TCH)
	n = 10	n = 14	n = 10
Age (years)	26.41	26.58	25.11
Body weight (kg)	54.31	45.87	43.50
Patients having previous attack of malaria	-	9(64.3%)	6(60%)
Haemoglobin (gram percent)	12.8	10.5	10.2
Initial parasite count (<i>P. falciparum</i> per microlitre)	-	5178.9	8791.8
Parasite clearance time (hours)	-	58.5	47.4
Fever clearance time (hours)	-	36.2	39.6
Recrudescence	-	1	-
Adverse effects	-	-	-

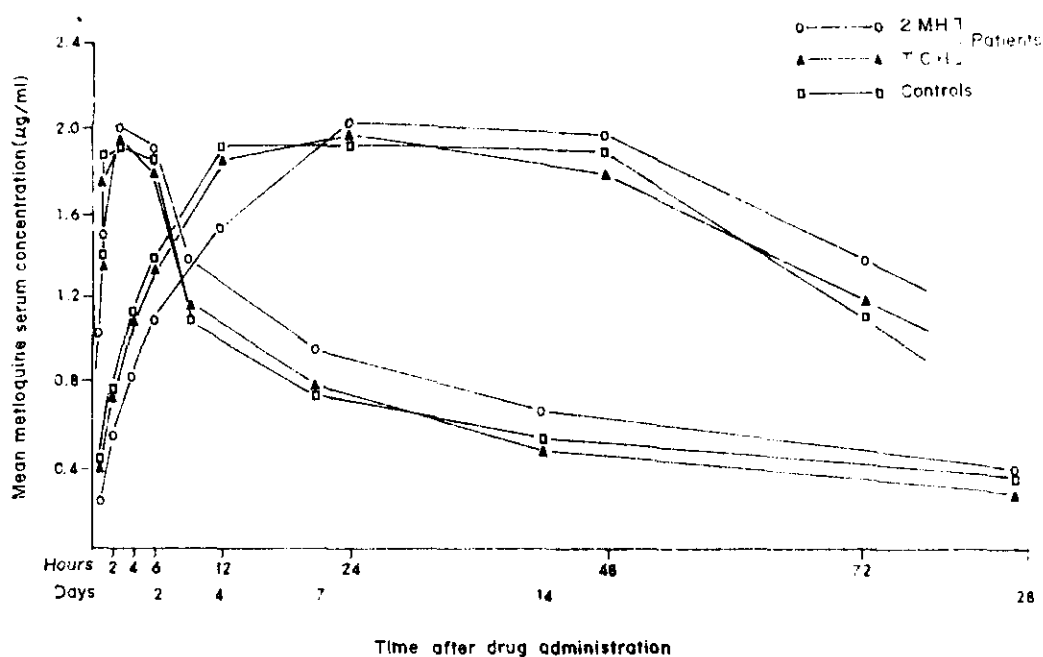


Fig. 1. Mean serum concentration-time profile of mefloquine in different groups

relatively rapid, having the absorption half-life of only 6.38 ± 0.6 hours, reaching the maximum serum concentration of 2.24 ± 0.05 $\mu\text{g/ml}$ at approximately 30 hours after drug administration.

Table 2 shows the mean pharmacoki-

netic parameters of mefloquine in the 3 groups studied. The rate of absorption was slower in malaria patients than in healthy volunteers and this resulted in a longer time to reach the peak serum concentration, but the difference is not statistically

Table 2. Mean pharmacokinetic parameters of mefloquine in different groups studied

Groups	Pharmacokinetic parameters							
	$T_{1/2ab}$ (hours)	$T_{1/2\alpha}$ (days)	$T_{1/2\beta}$ (days)	T_{max} (hours)	C_{max} (ug/ml)	AUC (ug/ml/hr)	Cl/f (L/kg/hr)	Vd/f (L/kg)
Healthy volunteers (n = 10)	4.87	1.25	18.63	25.68	2.27	451.32	0.029	18.68
Patients with <i>P. falciparum</i> malaria								
(a) Group A (No. 2, Military Hospital n = 14)	6.58	1.60	15.38	31.68	2.27	545.65	0.028	14.91
(b) Group B (Tharyawaddy Civil Hospital n = 10)	6.09	0.81	14.41	31.20	2.21	448.50	0.039	19.46

Table 3. Pharmacokinetics of mefloquine in healthy volunteers and falciparum malaria patients

Reference	Race	Dose	$T_{1/2ab}$ (hours)	$T_{1/2el}$ (days)	T_{max} (hours)	C_{max} (ug/ml)	Vd/f (L/kg)	Cl/f (L/kg/hr)
<u>Healthy volunteers</u>								
Desjardins et al. (1979) (n = 4)	Caucasian	1000 mg	3.80	13.9	19.6	0.800	13.3	0.066
Karbwang et al. (1986) (n = 6)	Thai	750 mg	-	17.8	20.0	1.309	18.5	0.026
Manson et al. (1985) (n = 10)	Malasian	500 mg	4.30	16.1	5.7	1.010	17.6	0.07
Schwartz et al. (1982) (n = 13)	African	1000 mg	1.53	20.0	-	1.020	18.2	0.025
Thaw-Zin et al. (1993) (n = 10)	Myanmar	1000 mg	4.80	18.6	25.7	2.207	18.7	0.029
<u>Falciparum malaria patients</u>								
Karbwang et al. (1987a) (n = 9)	Thai	750 mg	4.90	-	22.7	1.815	-	-
Karbwang et al. (1988b) (n = 6)	Thai (female)	750 mg	13.40	15.8	18.0	1.631	-	-
Karbwang et al. (1988a) (n = 12)	Thai	750 mg	-	12.2	-	1.789	15.7	0.014
Juma & Ogeto (1989) (n = 12)	Kenyan	750 mg	-	20.5	24.0	0.530	30.8	0.053
Thaw-Zin et al. (1993) (n = 24)	Myanmar	1000 mg	6.39	14.9	31.4	2.241	17.5	0.034

significant. The remaining parameters were more or less similar in both healthy volunteers and malaria patients.

Table 3 shows the comparison of mean pharmacokinetic parameters of Myanmar subject with those reported by various authors. Myanmar subjects 7 (i.e. both patients and normal subjects) were shown to have a slower rate of absorption and a lower volume of distribution, but the maximum serum concentration reached was much higher than those reported by other authors. The remaining parameters, however, fell well within the range previously reported.

DISCUSSION

Mefloquine is a highly effective antimalarial drug and cure rates obtained in the initial field trials had been close to 100% [3-5]. Since 1982, emergence of resistant strains to mefloquine became increasingly reported [10-11] and recently, susceptibility of some falciparum strains in the Thai-Cambodian border has decreased to such an extent that even adequate mefloquine concentrations in the blood were insufficient to clear parasitaemia [12,13]. Also in Myanmar, a decreased efficacy of mefloquine in uncomplicated falciparum malaria, in spite of adequate serum mefloquine levels, has been reported by Ye Thwe *et al.* in 1992. Since therapeutic failure or success is influenced by factors such as: (1) the immune status of the host (2) the susceptibility of the parasite to the drug and (3) the pharmacokinetic properties of the drug, this study was conducted with the aim to understand the influence of pharmacokinetic parameters on the therapeutic outcome in patients with uncomplicated falciparum malaria patients in Myanmar.

The findings in the present study

show that mefloquine was rapidly absorbed, reaching the mean MIC level of 0.32 ug/ml (0.15-0.6 ug/ml) [14] within 2 hours after drug administration and persisted well above this level up to 4 weeks and this may have resulted in an excellent therapeutic response seen in the study. An extremely prolonged terminal elimination half-life makes mefloquine an ideal drug for single curative dose therapy or once weekly prophylactic therapy, but it also increases the likelihood of inducing rapid development of resistance since it remains in the blood at subtherapeutic levels for extended period of time after day 28. This prolonged elimination half-life may be due to the large volume of distribution and a very low total clearance of mefloquine seen in the study.

Although the study shows a slower rate of absorption of mefloquine in malaria patients than in healthy controls, no difference was seen in the bioavailability (i.e. the extent of absorption as indicated by the AUC) and the maximum serum concentration reached. A similar finding was reported by Boudreau [8] who found a decreased rate of absorption in malaria patients without any alteration in the extent of absorption, but he also reported a higher C_{max} in malaria patients which was not seen in the present study.

Comparative pharmacokinetics between patients from different countries shows a wide range of variation which may be related to the ethnic origin of the patients (i.e. the pharmacogenetic difference) [15] but it may also reflect the difference in the brand of drugs used (i.e. bioavailability) [16] or changes secondary to the severity of malaria infection which is influenced by the immune status of the host and the susceptibility of the parasite to the drug, both

which may vary from place to place. The higher C_{max} seen in Myanmar patients may reflect a lower body weight of Myanmar subjects. Since 1000 mg mefloquine was found to be well tolerated and serious adverse effects were not reported, it can, thus, be taken as an acceptable therapeutic dose.

Finally, the finding that one patient from the No. 2 Military Hospital relapsing after 28 days (R1) while his mefloquine concentration in the serum was maintained well above the MIC (0.42 ug/ml) may indicate an emergence of mefloquine resistance in Myanmar.

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Essential element contents of surface and artesian well water in Yangon area

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A total of 91 samples of water collected from 18 townships within Yangon area comprising 31 numbers of surface wells and 60 numbers of artesian wells were chemically examined. All samples were determined for their concentrations of elements such as iodine, calcium, magnesium, iron, copper and zinc with the corresponding depths of wells. Average iodine contents of water was found to decrease as the depth of well increased. Magnesium concentration was also observed in the same manner as iodine. Calcium concentration was more in surface well water and no difference among artesian wells water. There was no relationship between calcium concentrations and the depths of the wells.

INTRODUCTION

Drinking water is one of the important natural sources which may contribute some amounts of essential elements such as iodine, calcium, magnesium, iron, copper and zinc for human nutrition. Since there was no report on the concentration of those elements of water in relation to depth of wells, the present study was conducted to reveal the essential element contents depending on the depths of wells. The contribution of essential elements from such drinking water as a natural source should be known to notify how much water can supply the requirement of those elements for human health.

MATERIALS AND METHODS

Ninety one samples of water of 31 surface wells and 60 artesian wells were analysed in this study. Those samples were collected from 18 townships namely Bahan, Sanchaung, Kamayut, Tamwe, Kyimyindine, Hlaing, Yankin, Tharkayta, Dawbon, Thanlyin, Hlinethaya, Shwepyitha, Thingangyun, Insein, Htaukkyant, Mayangon, South Okkalapa and Dagon Myo-thit.

Iodine content was estimated by photometric method [1]. Transmittance measurement at λ_{max} 525 nm was done by UV-VIS spectrophotometer (model DU-50, BECKMAN). Other mineral concentrations were determined by atomic absorption spectrophotometer (model SP-9 PYE UNICAM). Samples were classified into five groups according to the depths of wells as shown in tables and figures. Average concentration of each element for each group was determined and compared.

RESULTS

Average iodine concentrations of samples with the corresponding depths of wells are shown in Table 1. A decrease in the iodine content of water as the depth of well increases can be seen in Figure 1.

Regarding the mineral concentrations of water, iron was not detected in all samples, and zinc and copper were detected as trace amounts in a few samples (Table 2). The changes of concentrations of calcium and magnesium with the depth of well are described in Figures 2 and 3

respectively.

Table 1. Average iodine contents of surface and artesian well water

	Depth (feet)	Iodine content (Ug/l)	
		Range	Average
Surface wells	12 - 60	1.64 - 90.8	23.2 (n = 31)
Artesian wells	61 - 100	N.D. - 134.4	22.3 (n = 21)
Artesian wells	101 - 200	N.D. - 100.0	10.4 (n = 25)
Artesian wells	201 - 400	N.D. - 22.4	4.8 (n = 9)
Artesian wells	401 - 630	N.D. - 2.5	0.9 (n = 5)

N.D. = Not detected

n = Number of wells

Table 2. Mineral concentrations of surface and artesian well water

Wells & depths (feet)	Calcium Ca (ppm)	Magnesium Mg (ppm)	Iron Fe (ppm)	Zinc Zn (ppm)	Copper Cu (ppm)
Surface wells (n = 31) (12 - 60)	1.4 - 40.9 (8.7)	0.6 - 10.6 (5.6)	N.D.	N.D. - 0.2	N.D. - 0.03
Artesian wells (n = 21) (60 - 100)	0.3 - 4.8 (1.8)	0.1 - 10.1 (4.1)	N.D.	N.D. - 0.05	N.D. - 0.02
Artesian wells (n = 25) (101 - 200)	0.1 - 6.8 (2.1)	0.2 - 8.5 (3.4)	N.D.	N.D.	N.D. - 0.02
Artesian wells (n = 9) (201 - 400)	0.3 - 3.7 (1.8)	0.2 - 3.2 (2.7)	N.D.	N.D.	N.D. - 0.02
Artesian wells (n = 5) (400 - 630)	0.9 - 2.6 (2.0)	0.3 - 4.7 (2.1)	N.D.	N.D.	N.D. - 0.02
W.H.O. Standards	75 - 500	50 - 150	0.1 - 1.0	5 - 15	0.05 - 1.50

N.D. = Not detected

n = Number of wells

DISCUSSION

The result of average iodine concentrations indicates that surface well water contains more iodine than artesian well water. Among artesian well water iodine content decreases as the depth of well increases. The cut off level of iodine in the natural drinking water is 10 ug/L [2,3]. According to the table 2 and figure 1, average iodine content of surface well water and shallow artesian well (less than 200') water is above the cut off level. However water of deep artesian wells (more than 200')

shows low level of iodine (4.8 and 0.9 ug/L).

Of the essential mineral elements determined in this study, only calcium and magnesium were detected as a certain amounts. Average calcium concentration of surface well water was more than that of artesian well water. But no significant change of calcium concentration with depth of artesian well water was observed. Average magnesium concentration was also found to be higher in surface well water when compared to artesian well water. A slight decrease in

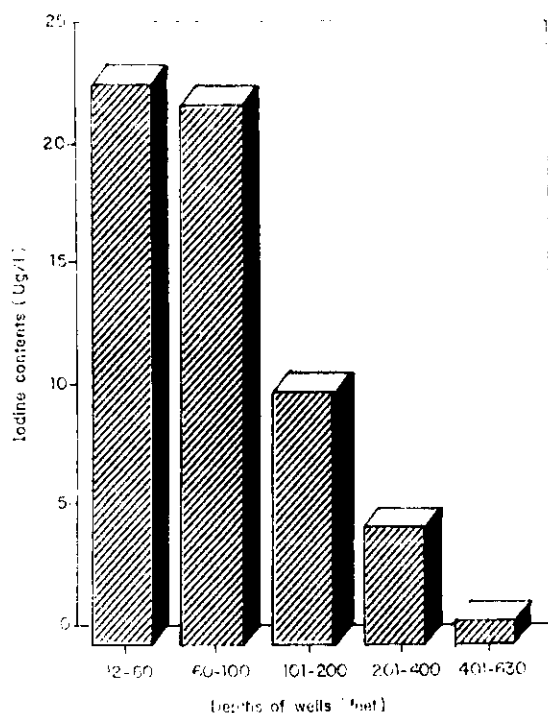


Fig 1. Average iodine contents of surface and artesian well water

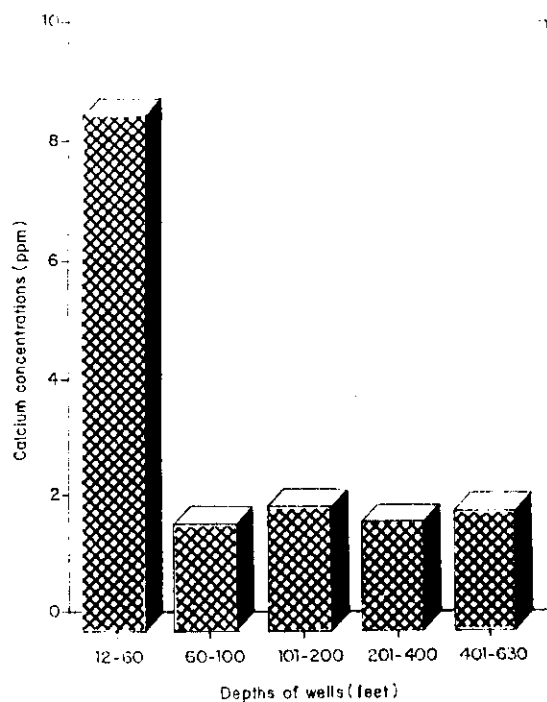


Fig. 2. Average calcium concentrations of surface and artesian well water

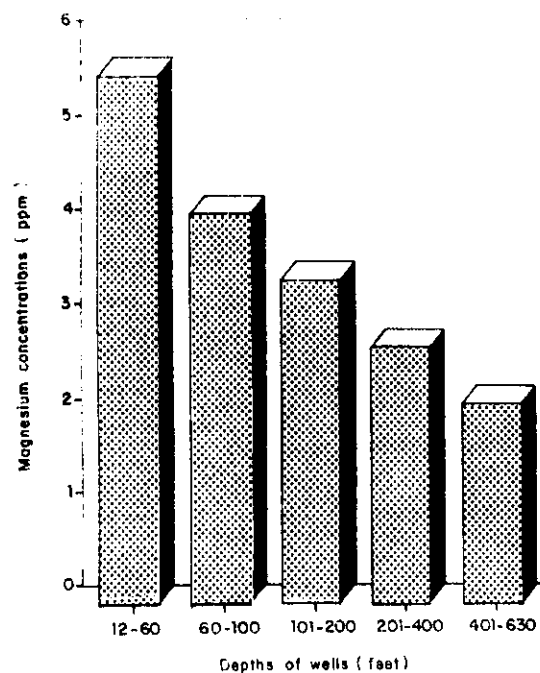


Fig. 3. Average magnesium concentration of surface and artesian well water

magnesium content of water was detected as the depth of artesian well increased.

As shown in table 2, all mineral concentrations of water show below the minimum desirable and maximum permissible concentration of each element of drinking water which had been recommended by World Health Organization [4].

This study has revealed that surface and shallow artesian well water can supply the iodine more than cut off level, 10 ug/L for human health but deep artesian well water cannot. All essential mineral contents of water of Yangon area were in lower level when compared to WHO standards.

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**Preparation of enteropathogenic *Escherichia coli* (EPEC) 'OK' and 'O' antisera
for identification of *E. coli* from diarrhoea cases**

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Standard strains of enteropathogenic *E. coli* (EPEC) were used to raise 'OK' and 'O' antisera in rabbits. The antisera obtained were 01k51, 026K60, 044K74, 055K59, 086K61, 086K62, 0111K58, 0114K90, 0119K69, 0125K70, 0126K71, 0127K63, 0128K67, 0142K+, 0146K89 and 0157K+. A total of 1805 isolates of *E. coli* isolated from 290 diarrhoea cases and 352 control cases were tested by 'OK' antisera by slide agglutination and it was found that 167 isolates showed agglutination. It was then serotyped by using 'O' antisera using test tube serial dilution technique and observed that 93 isolates showed agglutination.

INTRODUCTION

Since 1947, many investigators have studied the antigens of *E. coli* and as a result of their efforts, the numbers of delineated O, K and H antigens have been extended greatly. There are hundreds of serotypes of *Escherichia* and it is also apparent from many reports by Edwards & Ewing [1] that serotypes that belong to many O antigen groups occur commonly in the colon of normal human and lower animals. There are three general groups of serotypes that are capable of producing sporadic cases and outbreaks of diarrhoeal disease in humans, in other primates and in some species of lower animals. They are enteropathogenic *E. coli* (EPEC) enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and enterohaemorrhagic *E. coli* (EHEC). In the 1940s the application of serotyping detected certain serotypes (e.g. 0111 and 0125) more frequently from infants with diarrhoea than from healthy controls. However, not until 1978 was a causal relation unequivocally demonstrated in studies in human volunteers. Although these strains induce a watery diarrhoea not

unlike that seen in ETEC strains, EPEC do not produce either LT or ST, nor do they possess the same colonization factors as in ETEC.

EPEC-induced diarrhoea in the rabbit is correlated with histopathologic lesions identical to that seen in biopsies from infant babies. The capacity of EPEC strains to adhere tightly to cultured human cells is frequently associated with a plasmid. The precise nature of the EPEC adherence factor (EAF) is not known; it does not appear to be fimbriae. EPEC strains have also been found to elaborate cytotoxin that is enterotoxic and is similar to a toxin synthesized by some *Shigella* spp. Evidently, the pathogenesis of EPEC involves a specialised adhesion followed by synthesis of sufficient cytotoxin to have an enterotoxic effect. Thus EPEC remains an important cause of infant gastroenteritis and its pathogenic mechanism remains unclear. Various authors reported the usefulness of OKH serotyping of EPEC [2-5]. This paper also aims at the production of EPEC antisera for detecting *E. coli* from diarrhoea cases.

MATERIALS AND METHODS

Bacterial strains

The following EPEC standard strains (obtained from Centre for Disease Control, Atlanta, USA) were used for both 'OK' and 'O' antisera.

026K60 (H311b), 044K74 (H702C), 055K59 (Su3912/41), 0111K58 (stoke W), 0119K69 (W34), 0125K70 (Canioni 2745/53), 0127K83 (Holcomb 4932-53), 0142K+ (C771), 01K51 (U5/41), 086K61 (H35), 086K62 (Denka), 0114K90 (W26), 0126K71 (E611/6021/50), 0128K67 (Cigleris/56-54), 0146K89 (2950/54) and 0157K+ (A2).

Rabbits for immunization

Preparation of 'OK', 'O' antigen, immunization schedule (Table 1) and absorption of antisera were also done as described by Mar Mar Nyein *et al.* [6].

Serotyping of *E. coli*

The source of *E. coli* was from diarrhoea cases of children under five years of age. The isolates were tested with OK polyvalent antisera by slide agglutination and further confirmed by monovalent antisera. Later the specific 'O' antisera was used in tube agglutination test to confirm the presence of specific somatic antigen of the *E. coli*.

RESULTS

The amount of antisera obtained ranged between 40 to 80 ml. The least titre of 'OK' antisera was 1:40 (Table 2) and the antisera so obtained was preserved with sodium azide. The least titre for 'O' antisera was 1:320 and the antisera so obtained was preserved with glycerol. The titre of 'O' antisera is shown in Table 3.

A total of 1805 isolates of *E. coli* from diarrhoea cases were serotyped

Table 1. Immunization schedule

Sr. no.	'OK'		'O'	
	Time (day)	Amount of antigen injected (ml)	Time (day)	Amount of antigen injected (ml)
1	1	0.5	1	0.5
2	5	1.0	5	1.0
3	9	2.0	9	2.0
4	13	3.0	13	3.0
5	17	4.0	17	3.0
6	24	bled	24	bled
	>1:320		>1:1280	
7	If <1:320	4.0(boost)	If <1:1280	3.0(boost)
8	day 31	bled	day 31	bled

firstly by 'OK' antisera by slide agglutination and confirmed by 'O' specific 'O' antisera by test tube agglutination. It was found that 167 isolates of *E. coli* agglutinated with one of the OK antisera. They were further tested by 'O' antisera by tube agglutination and 93 isolates showed specific 'O' antigens. The number of isolates and cases agglutinated by different serotypes are shown in Table 4. Out of 93 isolates it was found that 0126 was the most common serotype isolated from diarrhoea cases. The titre of 'O' antigen ranged from 1:40 to >1:640 (Table 5).

DISCUSSION

Serotyping for the identification of *E. coli* strains capable of causing enteric diseases has been applied mostly to the group of organisms known as enteropathogenic to humans. A limited number of the approximately 162 O serogroups of *E. coli* have been associated with outbreaks of diarrhoea in nurseries. Serotyping of *E. coli* strains is a complicated and difficult process and very seldom yields definitive aetiological informa-

Table 2. Homologous titre of 'OK' antisera using standard strains by slide agglutination

Antigen \ Antisera	Code Number	026	044	055	0111	0119	0125	0127	0142	01	086	086	0114	0126	0128	0146	0157
		K60	K74	K59	K58	K69	K70	K63	K+	K51	K61	K62	K90	K71	K67	K89	K+
026K60	H311b	80															
044K74	H702c		80														
055K59	Su3912/41			80													
0111K58	stoke W				80												
0119K69	W34					40											
0125K70	Canioni 2745/53						40										
0127K63	Holcomb 4932-53							40									
0142K+	C771								40								
01K51	U5/41									80							
086K61	H35										40						
086K62	Denka											80					
0114K90	W26												80				
0126K71	E611/6021/ 50													80			
0128K67	Cigleris/ 56-54														80		
014K89	2950/54															40	
0157K+	A2																80

[illegible]

Table 4. Number of isolates and cases agglutinated by EPEC 'O' serotypes

Serotypes	No. of isolates	No. of cases
026	7	5
044	4	3
086	1	1
0111	4	2
0119	12	9
0125	2	1
0126	60	30
0128	3	2
Total	93	53

genetic similarity among strains, the correlation with pathogenicity is high; and serotyping has been of extraordinary importance in distinguishing the small number of strains that actually cause disease. The capacity of EPEC strains to adhere tightly to cultured human cells, are frequently associated with the plasmid [9]. The precise nature of EPEC adherence factor (EAF) is not known and it does not appear to be fimbriae. The genes associated with EAF have been cloned and their use in DNA probe showed that they were res-

Table 5. Titre of 'O' antigen of *E. coli* isolated from diarrhoea cases

Serotypes	No. of isolates	1:40	1:80	1:160	1:320	1:640	>1:640
026	7					4	3
044	4				1	2	1
0111	4		1			2	1
0119	12					5	7
0125	2					2	
086	1						1
0126	60	3	1	3	1	32	20
0128	3		1	2			
Total	93	3	3	5	2	47	33

tion. Recently, a new enteric infection has been ascribed to *E. coli*. Haemorrhagic colitis is a distinctive form of severe, bloody diarrhoea that often required hospitalization [7]. *E. coli* 0157H7 strains have been implicated as the cause of this disease, and they can be definitely identified only by serotyping. Accurate identification of organisms causing serious enteric disease is of great value for choosing appropriate antimicrobial agents, for prognosis, for recognizing potential danger of contacts and for epidemiological investigation of the source of infection [8].

E. coli O_K and H antigens are usually determined by chromosomal genes. Although the serotypes do not necessarily provide an index of overall

restricted to serotypes implicated in worldwide EPEC outbreaks (055, 0111, 0119, 0127 and 0128). Full serotyping with over 160 O antisera and over 50 H antisera may reveal new EPEC serogroups and serotyping is always worthwhile in outbreaks of diarrhoea. The value of routine 'O' groups determined in sporadic cases is open to question since many strains of *E. coli* belonging to EPEC O group may not cause diarrhoea.

Enteropathogenic *E. coli* (EPEC) were the first group of diarrhoea-causing *E. coli* to be identified [10] and they remain an important cause of disease worldwide [11]. Despite considerable progress in the understanding of some *E. coli* diarrhoea syndromes, the pathogenesis of EPEC infection remains poorly defined. EPEC are distinct from other *E. coli*

that cause diarrhoea. Firstly, they belong to a restricted set of serotypes. Second, they do not produce the heat stable or heat labile enterotoxins of enterotoxigenic *E. coli* (ETEC). Third, unlike enteroinvasive *E. coli* (EIEC), they do not induce keratoconjunctivitis on inoculation into the conjunctival sac of guinea pigs (Sereny test) [12,13]. Although some EPEC produce high levels of shiga-like toxins, many do not [14]. EPEC can be distinguished by the characteristic ultrastructural intestine lesion they induce [15,16].

From this study the antisera so obtained are useful in serotyping the *E. coli* and hence beneficial in identification of *E. coli* from diarrhoea cases. In this study it is noted that 0126 is the most prevalent agent among the diarrhoea cases; 0119 also plays an important role in causing diarrhoea. It would be worthwhile to know the distribution of EPEC among the population. It is also noted that the titre of *E. coli* varies from one isolate to another and it might reflect the pathogenicity of *E. coli* and more study is needed for its virulence.

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The effect of some medicinal plants on uterine smooth muscle

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Some selected plants reputed for use in menstrual disorders were scientifically investigated for their efficacy. Plant aqueous extracts were preliminarily screened for the efficacy on rat's uterine smooth muscle. The effects of aqueous extracts of Eclipta alba (L.) Hassk. (Kyeik-hman), Amaranthus spinosus Linn. (Hinnu-nwe-subauk) and Averrhoa carambola Linn. (Zaung-yah) were tested in vitro using isolated rat's uterus smooth muscle. Kyeik-hman, Hinnu-nwe-subauk and Zaung-yah whole plant aqueous extracts significantly induced contraction on the uterine smooth muscle.

INTRODUCTION

The plant Amaranthus spinosus L. (Hinnu-nwe-subauk) (family Amarantaceae) is an erect spinous herb that grows in India, Ceylon and many tropical countries. It grows wildly in Maymyo, Yangon, Bhamo and Mergui [1]. The root is considered diuretic, laxative and galactagogue. The decoction is given for retention of urine and gonorrhoea. It is also heating, expectorant, lessens the menstrual flow and useful in leucorrhoea and leprosy [2]. It is used in memorrhagia and eczema; and as a poultice it is applied to bubose and abscesses for hastening suppuration. It is also considered a lactagogue and specific for colic. The boiled leaves and roots are given to children as a laxative [2].

The plant Averrhoa carambola L. (Zaung-yah) (family Oxilidaceae) is a small tree whose native is Malaya. It is cultivated throughout the tropics. It is planted in Yangon, Mandalay and grows wildly in Myitkyina [1]. In Cambodia the leaves are considered antipyretic and antipruritic. In Indochina the leaves are employed in the treatment of scabies; they are also

said to be anthelmintic [2].

The plant Eclipta alba (L.) Hassk. (Kyeik-hman) (family Compositae) is a common weed growing in moist situations all round the tropics. It was reported that, in Bombay, the natives use the juice in combination with aromatics, as a tonic and deobstruent, and give two drops of it with eight drops of honey to new-born children, suffering from catarrh. The plant cures inflammation, hernias, eye diseases, bronchitis, asthma, leucoderma anaemia, diseases of the heat and the skin, itching, night-blindness, syphilis, is used to prevent abortion and miscarriage, and for uterine pains after delivery [2].

It is also claimed that the plant has a bitter sharp taste, improves the colour of the hair and the lustre of the eye, tonic, expectorant, antipyretic, stomachic; good for diseases of the spleen, stomatitis, tooth ache, head ache, hemicrania, fever, internal disease, pain in the liver and cures vertigo [2].

It is primarily used as a tonic and deobstruent in hepatic and splenic enlargement, and in various chronic

skin diseases. The juice of the leaves is given in one teaspoonful dose in jaundice and fevers. The root is given to relieve the scalding of urine. The plant is much used as a cure for asthma and bronchitis in Indochina. The pounded leaves are prescribed in haemorrhage. In Ceylon, it is used to purify the blood [2]. Among the Malays decoction of the root is given to women after childbirth, either alone or with cummin seeds [4]. Liquid extract from fresh *E. alba* leaves proved to be effective *in vivo* in preventing carbon tetrachloride (CCl_4) induced liver damage in guinea pigs [5].

This study was designed to investigate the effect of the stated plant aqueous extracts on the uterine smooth muscle of rats.

MATERIALS AND METHODS

Plant collection and extract preparation

The plant materials used in this study were collected from Yangon area. The plants were identified and authenticated by a botanist and its identity was confirmed and deposited at the Pharmacognosy Section, Pharmacology Research Division, Department of Medical Research, Yangon, Myanmar. The whole plant was air-dried and powdered mechanically. Aqueous extracts were prepared by boiling 30 g dried powder with 300 ml distilled water for 6 hrs, then filtering twice through cheese cloth. The filtered solutions were then allowed to dry on a boiling water bath in petri dishes. The required concentrations for the *in vitro* tests were prepared dissolving substances in distilled water. Qualitative identification tests of the chemical constituents present in the effective tested samples were conducted according to the standard procedures as stated in

Trease and Evans [6] and Pharmacopoeia [7].

Preparation of isolated uterine horns

Female rats weighing 200-250 g were used. Animals were sacrificed and the uterine horns were then dissected from the animals. Each horn was vertically mounted in a 50 ml organ bath filled with De Jalon's solution. The perfusion solution was kept at 30°C and continuously bubbled with 95% O_2 and 5% CO_2 gas mixture. The preparation was left to equilibrate for at least 30 mins before starting the experiment. The animals were estrogen primed by subcutaneous injection of stilbesterol (0.1 mg/kg subcutaneously) 24 hrs prior to sacrifice [8]. Contractions and relaxation of the uterine muscle were recorded isometrically as 1 gm tension via a force displacement transducer (F-60 myograph, Narco-Biosystem) connected to a physiograph Narcotrace, (Narco-Biosystem). To determine the effect of oxytocin (0.0005 unit/ml) aqueous extracts of the above three plants on the uterine muscle, 0.5 ml of each extract (50 mg) and 1.0 ml of each extract (100 mg) were added to the organ bath immediately after the equilibration period. The influence of the test substances on the frequency and force of contraction was determined as described previously [9].

RESULTS

Qualitative tests of the aqueous extracts

The results from the qualitative identification tests on the effective aqueous extracts of three plants are shown in Table 1. As shown in the table, alkaloid, tannins and steroids were present in all the three plant extracts. Quinine was determined in

Table 1. Qualitative test of aqueous extracts of *A. spinosus* L. (Hinnu-nwe-subauk), *A. carambola* L. (Zaung-Yah) and *E. alba* (L.) Hassk.

Sample	Alkaloid	Quinone	Flavonoid	Tannins	Saponin	Triterpene steroid
1. Hinnu-nwe-subauk Aqueous extract	+	-	-	+	+	+
2. Zaung-yah Aqueous extract	+	+	-	+	+	+
3. Kyeik-hman Aqueous extract	+	+	+	+	-	+

Zaung-yah and Kyeik-hman aqueous extracts whereas flavonoid was detected only in Kyeik-hman aqueous extract. So also saponin was found to be present in Hinnu-nwe-subauk and Zaung-yah aqueous extracts respectively.

Effect on the isolated uterus

The standard drug oxytocin 0.0005 unit/ml induced significant contraction on the isolated rat uterus. So also, addition of Hinnu-nwe-subauk (1-3 mg/ml bath concentration) to the estrogen-dominated rat uterus caused a marked increase in rate and amplitude of the spontaneous contractions. Addition of the aqueous extracts induced uterine contraction in a dose-dependent manner (Fig. 1). It revealed a consistent and potent oxytocic like activity on the isolated rat uterus.

Similarly, the addition of Zaung-yah aqueous extract to the estrogen-dominated rat uterus caused a marked spasmogenic action. It also induced uterine contraction in a dose-dependent manner (Fig. 2).

Addition of Kyeik-hman aqueous extract also caused a spasmogenic action like the former two plants tested. It also induced uterine contractions in a dose-dependent manner (Fig. 3). The increase in force of contractions in all the three plant

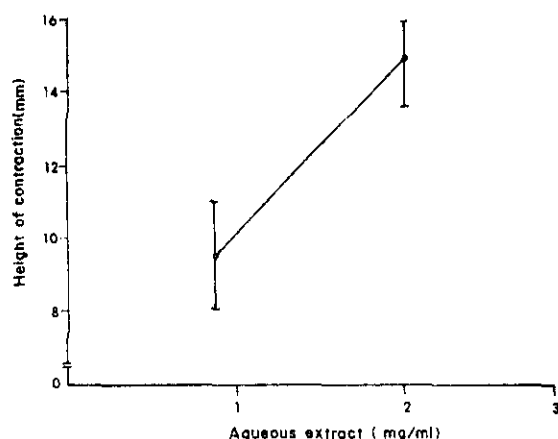


Fig. 1. Dose effect of Hinnu-nwe-subauk aqueous extract on the isolated rat's uterus model

The ordinate is the height of contraction in mm following the addition of aqueous extract. The abscissa indicates two dose levels of the extract added. Each point represents the means of 6-24 observations and vertical bars indicate standard errors of the means.

extracts were reversible.

DISCUSSION

Qualitative identification tests on the effective aqueous extract of the plant *A. spinosus* (Hinnu-nwe-subauk) show the presence of alkaloid, tannin, saponin and steroid. The plant *A. carambola* (Zaung-yah) contained alkaloid, quinone, tannin, saponin and triterpene steroid. The third effective

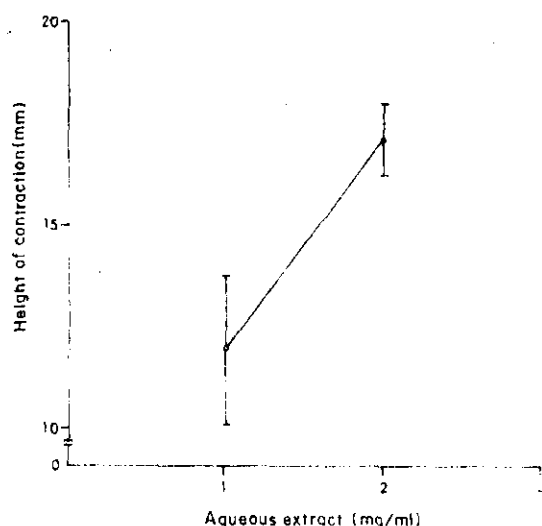


Fig. 2. Dose effect of Zaung-yah aqueous extract on the isolated rat's uterus model

The ordinate is the height of contraction in mm following the addition of aqueous extract. The abscissa indicates two dose levels of the extract added. Each point represents the means of 14 observations and vertical bars indicate standard errors of the means.

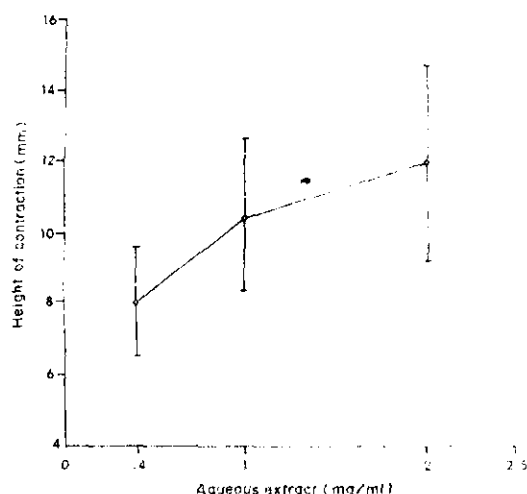


Fig. 3. Dose effect of Kyeik-hman aqueous extract on the isolated rat's uterus model

The ordinate is the height of contraction in mm following the addition of aqueous extract. The abscissa indicates various dose levels of the extract added. Each point represents the means of 6-20 observations and vertical bars indicate standard errors of the means.

aqueous extract of the plant *E. alba* (Kyeik-hman) contained alkaloid, quinone, flavonoid, tannin and triterpene steroid.

The plant Hinnu-nwe-subauk induced uterine contraction in a dose-dependent manner. Addition of Zaung-yah aqueous extract increased the frequency and height of contraction in a dose-dependent manner. It seemed to depend on the tissue variation. Among the tissues tested 67% of the tissue tested showed contraction and 33% showed relaxation. Addition of Kyeik-hman extract also produced increase in frequency and height of contraction. Addition of aqueous extracts to the isolated rat uterus induced contractions suggesting release of Ach and 5-HT and/or direct activation of the uterine muscarinic and serotonergic receptors. A similar mechanism may underlie the observed increase in the frequency of the uterine contractions. The results of this study outlines some of the oxytocic effects of some naturally occurring aqueous extracts of some plants. However we are in the process of attempting to isolate and identify the active constituents of the above three plants in order to determine the mechanism of action of these compounds which will be of use in certain menstrual disorders.

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Prevalence of hypoglycaemia in untreated patients with falciparum malaria

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Two hundred and four consecutive patients with *P. falciparum* malaria who attended Tharrawaddy Civil Hospital during 1990 and 1991 malaria seasons were studied. The factors such as age group, sex, parasite density, body temperature, presence of pregnancy and clinical categories of the patients were related to the plasma glucose level of the patients, 180 plasma samples from the patient's attendants and 54 plasma samples from patients with *P. vivax* malaria who attended to the same hospital were also included in the study as controls. High percentage of patients with hypoglycaemia (< 40 mg/dl) was present in patients with cerebral malaria, patients with high parasite density and women with pregnancy. But there was no significant difference between these groups of patients and controls. Hypoglycaemia in falciparum malaria may be multi-factorial and not due to single cause.

INTRODUCTION

There is a volume of literature on the association between hypoglycaemia and falciparum malaria. Patients with hypoglycaemia following quinine therapy had been repeatedly documented to be due to irritation of islets of Langerhans to produce more insulin in blood [1,2,3,4,5,6,7,8,9,10]. But there are also patients who are hypoglycaemic without quinine treatment.

Reports of hypoglycaemia in patients without quinine treatment

Moderately decreased blood sugar level in patients with falciparum malaria was first reported by Sinton and Kehr in 1931 [11]. Two cases was reported by Devakul (1960) [12]. In one the blood glucose concentration was only 4.3 mg per deciliter and at autopsy no glycogen was detectable in the liver. Migasena in 1983 had also reported patients with

hypoglycaemia before antimalarial therapy [13]. White et al. in 1987 reported hypoglycaemia developed in 15 of 47 Gambian children with falciparum malaria [14].

Prasarnphanich in 1987 also reported six out of forty-five children with cerebral malaria (13.33%) who were hypoglycaemic before antimalarial therapy [15]. Taylor et al. in 1988 has prospectively studied on 95 Malawian children with falciparum malaria and altered consciousness [16]. Nineteen patients were hypoglycaemic before treatment. Hypoglycaemia was associated with low plasma insulin concentrations and with elevated plasma concentrations of lactate, alanine and 5-nucleotidase. This finding suggests that impaired hepatic gluconeogenesis but not hyperinsulinemia contributes to the pathogenesis of pretreatment hypoglycaemia. A fatal case of hypoglycaemia in postpartum cerebral malaria was recorded by Currie and Kevau 1988 in Papua

New Guinea [17]. Taylor et al., 1990 also reported from Malawi describing hypoglycaemia in Tanzanian children with a variety of the illnesses other than malaria [18]. Thus the literature on hypoglycaemia on quinine untreated malaria patients is conflicting. Hence this study is needed.

Objectives

The objective of this study is to document the problem of hypoglycaemia among quinine untreated Myanmar patients with falciparum malaria and to find out the factors associated with it.

PATIENTS AND METHODS

Two hundred and four consecutive patients with *P. falciparum* malaria who attended Tharrawaddy Civil Hospital during 1990 and 1991 malaria seasons and who were free from quinine in urine as tested by Mayer TANRET-reagent were studied. The factors such as age group, sex, parasite density, body temperature, presence or absence of pregnancy, clinical categories of the patients were related to the blood glucose level of the patients. 180 plasma from the patient attendants and 54 plasma sample from patients with *P. vivax* malaria who attended to the same hospital during the same period were also included in the study as controls. The age group of the patients were recorded as 0-12, 13-20, 21-50, 51-60, and above 60 years groups. Parasite density of the patients was recorded according to the Bruce Chawatt Scale 1986 [19]. Staining and counting of malaria parasite was done according to WHO.

The body temperature of the patients were recorded at time of collecting of blood. Clinical categories of the patients were recorded as: (i) patients with no complications, (ii) patients with one of the complications such

as hyperparasitaemia (more than 5% of RBCs parasitized), jaundice, hyperthermia (more than 39°C), renal failure etc. and (iii) cerebral malaria patients with unrousable coma.

Blood samples were collected from the vein of the patients. Glucose estimation of the plasma was done at the same day of collection. Fluoride oxalate was used as anticoagulant to prevent the glucose from being destroyed in the blood. Blood glucose estimation was done by Othotoluidine method using photoelectric colorimeter.

RESULTS

The blood sugar level of 204 patients with *P. falciparum* malaria, (including 12 patients under 12 years) 54 patients with *P. vivax* and 180 controls were studied. There was no significant difference in the mean sugar level of patients and controls. But if blood sugar level of 40 mg/dl was taken as the demarcation line for hypoglycaemia (WHO, 1991) [20] the prevalence of hypoglycaemia was also not significantly different.

When the level of blood sugar and the prevalence of hypoglycaemia of the patients with *P. falciparum* were compared with patients with *P. vivax* infection there was no statistical difference between the two groups. There was also no statistical difference between males and females and there was no preponderance of hypoglycaemia in any age group and in a specific body temperature group.

When the mean blood sugar level belonging to different clinical category of the patients were compared, there was no significant difference among the different sub-groups. But there was a trend in the prevalence of hypoglycaemia to be high among patients with cerebral malaria (Table 1).

Table 1. Blood sugar level (mg/dl) in different clinical categories of patients

Category group	No. studied	Mean & standard deviation (mg/dl)	Hypoglycaemia	
			No.	%
Cat I	119	81.54±24.32*	4**	3.36
Cat II	50	85.57±23.24*	2**	4
Cat III	35	82.40±36.54*	4**	11.42

* No difference between the different groups

** > P > 0.02

When the mean blood sugar level belonging to different parasite density were compared, there was no significant difference among the different sub-groups, but there was a trend in the prevalence of hypoglycaemia to be high among the patients with high parasite density (Table 2).

Table 2. Blood sugar level (mg/dl) in different parasite density of patients

Parasite density*	No. studied	Mean & standard deviation (mg/dl)	Hypoglycaemia	
			No.	%
+	95	79.77±22.5**	3***	3.1
++	42	98.67±28.1**	1***	2.3
+++	32	90.80±24.35**	2***	6.25
****	35	76.80±25.28**	4***	11.42

* Bruce Chwatt scale

*** No significant difference between

Among women patients with *P. falciparum* malaria women with pregnancy tend to have lower level of blood sugar. There were only 12 patients with pregnancy and malaria. Sample size was small for statistical analysis (Table 3).

DISCUSSION

Hypoglycaemia is increasingly recog-

Table 3. Blood sugar level (mg/dl) in women with pregnancy & no pregnancy

Women	No. studied	Mean & standard deviation (mg/dl)	Hypoglycaemia	
			No.	%
Pregnancy	12	55.50±22.5 *	3**	25
No-pregnancy	54	81.78±29.66*	4**	7.4

** No significant difference between pregnant & non-pregnant women

nized as an important manifestation of falciparum malaria.

In this study ten out of two hundred and four quinine untreated *P. falciparum* malaria patients were associated with hypoglycaemia. Such patients with hypoglycaemia (1 out of 59) were also reported among the patients suffering from *P. vivax*. Thus hypoglycaemia in malaria may be due to fever and avoidance of food by the patients and not specifically to presence of *P. falciparum* infection alone. Hypoglycaemia with low insulin level had also been observed in African children with severe malaria as well as in adults before they are treated with quinine [3,10]. Thus fasting of food with associated glycogen depletion of impaired gluconeogenesis may be a factor for the cause of hypoglycaemia.

In this study a high number of patients with hypoglycaemia were recorded among those who were brought to hospital with cerebral malaria. This could be also due to the fact that patients cannot take food while they were unconscious.

In literature children had been reported to be associated with hypoglycaemia [14,15] but in this study hypoglycaemia was not common among children patients. The difference may be due to the fact that in this study there were only 5 patients

under 5 years of age. More children patients are needed to be studied.

In this study a number of patients with high level of parasites were commonly associated with hypoglycaemia. The fall in blood glucose level may be due to high utilization of the parasites. Glucose consumption increases in fever and infection [21]. Thus increased glucose turnover in acute falciparum malaria may also be attributed to increased glucose consumption by both host and parasite.

In this study pregnancy may be the factor that is associated with hypoglycaemia. White [3] and Looareesuwan [10] had also reported hypoglycaemia to be common in pregnant women with severe falciparum malaria. In these patients hypoglycaemia is commonly asymptomatic. Thus in all patients with pregnancy, blood sugar level should be determined daily while they are in hospital. To summarize, hypoglycaemia in falciparum malaria may be multifactorial and not due to a single cause.

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Experimental screening for anti-peptic ulcer activity of some Myanmar traditional medicine formulations

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Four Myanmar traditional medicine formulations (TMFs), namely TMF-02, TMF-03A, TMF-03B and TMF-08, locally claimed to be useful in treating dyspepsia, were screened for anti-peptic ulcer activity employing two experimental *in vivo* test models in rats. Only TMF-02, TMF-03B and TMF-08 markedly reduced ulcer severity to the ulcer indices of 17.4, 15.6 and 14.5 respectively. These effects were comparable to cimetidine (13.8), while that of the negative control was 35. Measuring gastric juice acidity in rats employing pyloric ligation confirmed that these three drugs did reduce gastric acidity, particularly diminishing the free acid one-third and shifting gastric pH from 6 to 7.

INTRODUCTION

Many traditional medicine formulations (TMFs) of Myanmar are used at traditional medicine hospitals and dispensaries for the treatment of peptic ulcer, a common ailment in this country as stated by the traditional medicine council [1]. A major sector of the population relies on these drugs because they are easily available for such a chronic regimen of treatment. The present study aimed at evaluating four traditional medicine formulations used widely in traditional practice to treat peptic ulcers. The drugs were TMF-02, TMF-03A, TMF-03B and TMF-08.

MATERIALS AND METHODS

Preparation of traditional medicine formulation (TMF)

The four Myanmar traditional drug formulations were prepared according to the methods employed by authorized personnel of the Indigenous Medical Council of Myanmar as listed in Tables 1, 2, 3 & 4 respectively. The

formulations were all in a powder-mixture form. All the single ingredients contained in the respective TMFs were market samples and were catalogued in the Crude Drug Museum of Department of Medical Research, Yangon, Myanmar. Many ingredients listed with Myanmar names were taxonomically identified for the botanical names as stated in Hundley and Chit Ko Ko [2] and San Khin [3].

Anti-peptic ulcer screening

The underlying principle was to screen the inhibitory action on peptic ulcer formation by using the method of Gupta *et al.* [4]. Following 24 hours fasting, each individual rat weighing 80-130 g was administered orally with either the test drug (1.6-3.2 g/kg) or cimetidine (100 mg/kg) or no drug. Each treatment group comprised 8-10 animals. One hour later, the ulcerogenic agent acetylsalicylic acid (aspirin) was administered orally to every animal, 300 mg/kg. At the end of 3 hour period of drug interaction, each animal was

Table 1. Ingredients of Laymyoshitsai hsay (TMF-02)

Scientific/Common names	Family	Myanmar name	Part used	Amount (g/100g)
Major ingredients:				
1. Natural sodium chloride; rock salt	-	Theindaw	-	45.9
2. Ammonium chloride	-	Zawetthar	-	22.9
3. Black salt*	-	Hsabokey	-	11.5
4. <u>Glycyrrhiza glabra</u> L.	Papilionaceae	Nweigyo	Stem	10.1
5. <u>Camphor</u>	-	Payoke	-	5.9
Minor plant ingredients:				
6. <u>Anacyclus pyrethrum</u> DC.	Compositae	Kutkarar	Root	1.4
7. <u>Syzygium aromaticum</u> (L.) Merr. & Perry	Myrtaceae	Layhnyin	Flower	1.4
8. <u>Elettaria cardamomum</u> Maton. var. <u>miniscula</u>	Zingiberaceae	Pharlarngei	Seed	0.9

*Baked sodium chloride mixture consisting of sodium, magnesium and iron sulphides

Table 2. Ingredients of Hsah setdabah hsay (TMF-038)

Scientific/Common names	Family	Myanmar name	Part used	Amount (g/100g)
Mineral/Salt ingredients;				
1. Crude sodium carbonate	-	Sithsatpyar	-	14.6
2. Potassium nitrate	-	Yanzein	-	14.6
3. Black salt*	-	Hsabokey	-	10.4
4. Magnesium sulphate	-	Bingali hsakhah	-	10.4
5. Natural sodium chloride; rock salt	-	Theindaw	-	10.4
6. Potassium aluminium sulphate; alum	-	Kyaukchin	-	10.4
7. Sodium bicarbonate	-	Hsawdar	-	10.4
8. Ammonium chloride	-	Zawetthar	-	9.4
9. Calcium oxide	-	Maphaukhton	-	3.1
Plant ingredients:				
10. <u>Myristica fragrans</u> Houtt.	Myristicaceae	Zardakpho	Fruit	3.1
11. <u>Zingiber officinale</u> Roscoe.	Zingiberaceae	Gyingyauk	Rhizome	3.1

*Baked sodium chloride mixture consisting of sodium, magnesium and iron sulphides

sacrificed by initial ether inhalation followed by cervical venesection. A mid-line abdominal incision was made to extricate the stomach, which was cut open along the greater curvature. The gastric mucosal

lining was examined for evidence of ulceration, using a magnifying lens of 2.5 times. The number of ulcers and the severity of each were recorded, the latter ranked from 1+(2mm); 2+(3mm); and 3+(4mm)

Table 3. Ingredients of Thwaydular hsay (TMF-08)

Scientific/Common names	Family	Myanmar name	Part used	Amount (g/100g)
Major ingredients:				
1. Unidentified	Unidentified	Hsaypoedi	Root	29.7
2. <i>Nigella sativa</i> L.	Ranunculaceae	Samonnet	Seed	20.0
3. Ammonium chloride	-	Zawetthar	-	20.0
4. Natural sodium chloride; rock salt	-	Theindaw	-	15.2
5. <i>Terminalia citrina</i> Roxb.	Combretaceae	Kyazoot	Fruit	7.6
Minor plant ingredients:				
6. <i>Acorus calamus</i> L.	Araceae	Linlay	Rhizome	0.3
7. <i>Alpinia galanga</i> Willd.	Zingiberaceae	Padegawgyee	Rhizome	0.3
8. <i>Anacyclus pyrethrum</i> DC.	Compositae	Kutkarar	Root	0.3
9. <i>Anethum sowa</i> Roxb.	Umbelliferae	Samonnyo	Fruit	0.3
10. <i>Cinnamomum zeylanicum</i> Breyn.	Lauraceae	Thitgyaboe	Bark	0.3
11. <i>Cinnamomum</i> sp.	Lauraceae	Karaway	Leaf	0.3
12. <i>Cuminum cyminum</i> L.	Umbelliferae	Ziyar	Leaf	0.3
13. <i>Elettaria cardamomum</i> Maton.	Zingiberaceae	Pharlarngei	Seed	0.3
14. <i>Foeniculum vulgare</i> Mill.	Umbelliferae	Samonsabah	Fruit	0.3
15. <i>Foeniculum vulgare</i> Mill	Umbelliferae	Samonphwe	Stem	0.3
16. <i>Glycyrrhiza glabra</i> L.	Papilionaceae	Nweigyo	Stem	0.3
17. <i>Lepidium sativum</i> L.	Cruciferae	Samonni	Seed	0.3
18. <i>Mesua ferrea</i> L.	Guttiferae	Gantgaw	Stamen	0.3
19. <i>Myristica fragrans</i> Houtt.	Myristicaceae	Zardeikpho	Fruit	0.3
20. <i>Picrorhiza kurroa</i> Royle	Gentianaceae	Hsaungmaygah	Root	0.3
21. <i>Piper longum</i> L.	Piperaceae	Peikchinn	Fruit	0.3
22. <i>Piper nigrum</i> L.	Piperaceae	Ngayokkoun	Fruit	0.3
23. <i>Saussurea</i> sp.	Compositae	Pannoot	Root	0.3
24. <i>Syzygium aromaticum</i> (L.) Merr & Perry	Myrtaceae	Layhnyin	Flower	0.3
25. <i>Trachyspermum ammi</i> L. Sprague	Umbelliferae	Samonbyu	Fruit	0.3
26. <i>Zingiber officinale</i> Roscoe	Zingiberaceae	Gyin	Rhizome	0.3
27. Unidentified	Unidentified	Panma	Bark	0.3
28. Unidentified	Unidentified	Pharlarnabeik	Fruit	0.3
29. Unidentified	Unidentified	Karaway	Fruit	0.3
Minor mineral/Salt ingredient				
30. Borax	-	Letchah	-	0.3

Table 4. Ingredients of Hsah ngabah hsay (TMF-03A)

Scientific/Common names	Myanmar name	Amount (g/100g)
Mineral/Salt ingredients:		
1. Ammonium chloride	Zawetthar	20
2. Black salt*	Hsaboke	20
3. Magnesium sulphate; epsom salt	Myanmar hsakhah	20
4. Natural sodium chloride; rock salt	Theindaw	20
5. Sodium chloride	Hsah yoeyoe	20

*Baked sodium chloride mixture consisting of sodium, magnesium and iron sulphides

respectively. The ulcer index (UI) was determined for each group by using the following formula:

$$UI = \frac{A_u}{A_t} + U + S$$

where A_u = percentage of animals showing ulcer

A_t = number of animals tested

U = average number of ulcers per stomach

S = average severity of ulcers

Effects on gastric acidity

The underlying principle was to determine the anti-hyperacidity action by employing the method of Shay et al. [5]. Rats of either sex (120-150 g) were fasted for 48 hours. Each animal was orally administered with one of the test drugs (TMF-02, 1.6 g/kg; TMF-03A, 1.6 g/kg; TMF-08, 3.2 g/kg) or cimetidine (100 mg/kg) or no drug. Each treatment comprised of 8-10 animals. One hour later, the abdomen was opened under light ether anaesthesia. Through a small midline incision, the pyloric portion of the stomach was identified and lifted out of the abdomen and ligated. The stomach was then ligated and replaced carefully back. The animals

were deprived of both food and water during the post-operative period for four hours. After which, the stomach was dissected. The contents were drained into a tube to determine the volume, pH, free and total acid contents.

RESULTS

Effects on aspirin-induced ulcer formation in rats

Out of the four TMFs tested, three drugs clearly showed anti-ulcer properties. The three drugs namely TMF-02, TMF-03B and TMF-08 markedly reduced the formation of gastric ulcers, resulting in ulcer indices of 17.4, 15.6 and 14.5 respectively, compared to 13.8 of the cimetidine positive-control group and to 35 of the negative-control group. TMF-03A was found to be ineffective resulting in the ulcer index of 30.7. Ulcer severity of these drugs were also noted as shown in Fig. 1.

Effects on gastric secretory activity in pylorus-ligated rats

Free-acid concentrations of the TMF-02, TMF-03B and TMF-08 showed a significant decrease ($p < 0.005$) similar to that of cimetidine ($p < 0.005$) when compared to that of the control (Fig. 2), whereas only TMF-02 and TMF-03B showed a significant decrease in total acid ($p < 0.005$; $p < 0.05$) similar to that of cimetidine ($p < 0.025$). The overall pH status indicated TMF-02 and TMF-08, like cimetidine, caused reduced acidity, diminishing especially the free-acid levels with a concomitant rise of the gastric pH ($p < 0.005$). In regard to drug effect on gastric juice volume, TMF-02 showed a marked increase in volume, due to the enormous bulk of the ingredients.

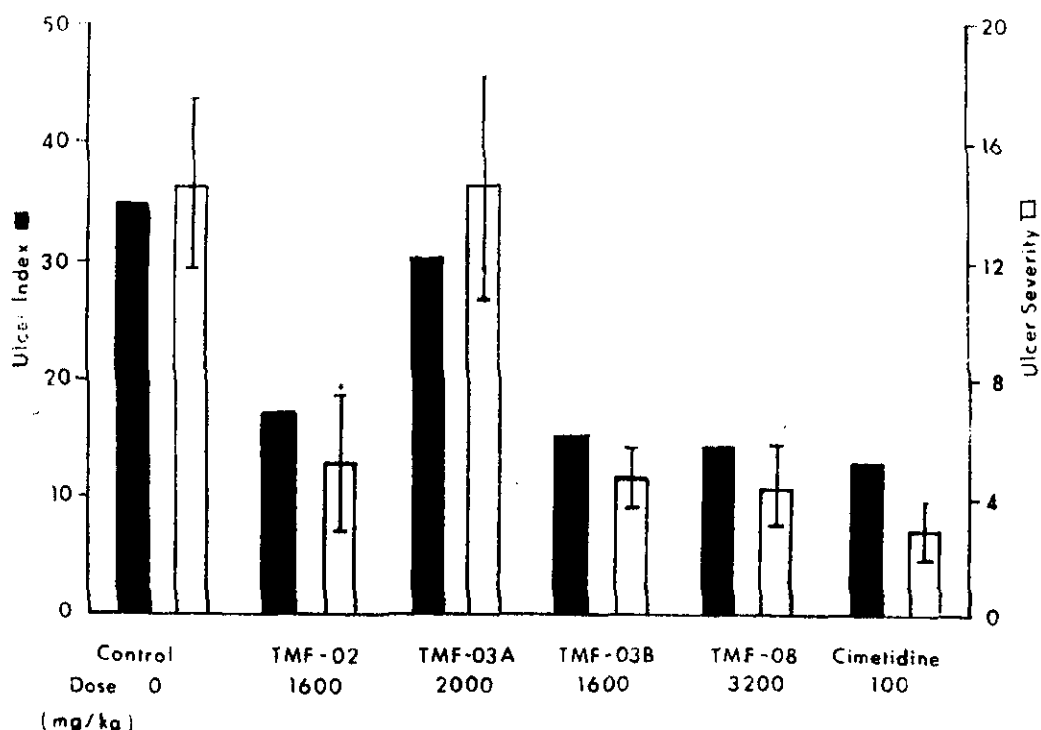


Fig. 1. Oral anti-ulcer effects of traditional medicine formulations (TMFs) on aspirin-induced peptic ulcer in rats. Each column represents the mean $\pm$ SEM response of 9-10 animals, which were administered with single dose of a TMF or cimetidine or no drug.
* $p < 0.005$ (significantly different from that of control group)

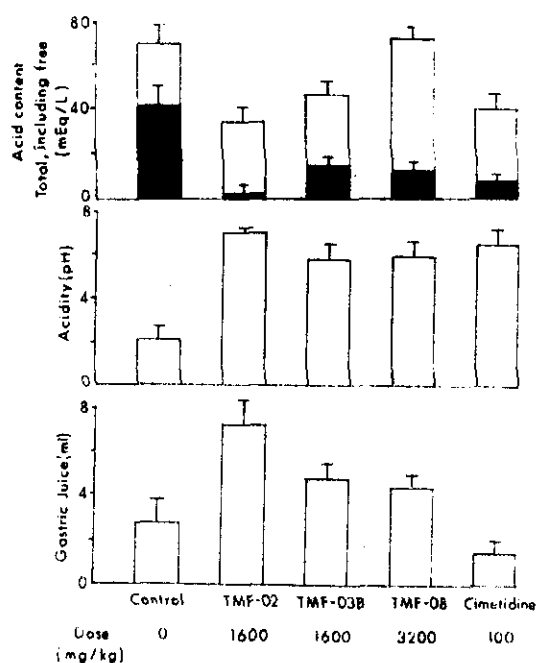


Fig. 2. Anti-secretory effects of traditional medicine formulations (TMFs) in pylorus-ligated rats. Each column represents the mean $\pm$ SEM response of 8-10 animals, which were orally administered with single dose of a TMF or cimetidine.

DISCUSSION

Out of the four TMFs investigated, only three of these namely, TMF-02, TMF-03B and TMF-08 showed positive results in reducing the ulcer index in rats with aspirin-induced ulcer. The results obtained were comparable to those seen with the clinically established anti-ulcer drug cimetidine. The fact that TMFs dosage employed were over ten times higher was due to the fact that they were basically non-refined. In terms of the average severity of ulcer, a significant reduction in ulcer severity was seen compared to that of the control group, except for TMF-03A.

Employing the pylorus-ligation method, it was found that the above mentioned three formulations effectively reduced gastric acidity. Free acid as well as total acid levels were reduced and the pH rose from 6 to 7, showing promising antacid properties. In

contrast to the previous Aspirin induced method, the average severity of ulcers in the ligation method did not show significant antisecretory activities.

The three traditional medicine formulations TMF-02, TMF-03B and TMF-08 showed promising pharmacological actions, the last was the most outstanding. The mechanism of action was suggested to be purely antacid, i.e., by direct neutralisation.

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Nutritional status and intestinal parasitosis in school-enrolled and non-enrolled school-age children in Yangon

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In Myanmar, over 38 percent of children in the age-group 5-9 years were never enrolled in school in 1990. Also, there is a lack of information on the comparative prevalence of malnutrition and intestinal parasitosis among school-enrolled and non-enrolled school-age children in Myanmar. We, therefore, undertook a cross-sectional survey comprising 3325 school children from 13 primary schools and 164 non-enrolled school-age children from neighbouring quarters in Tharkayta and Mingaladon townships of Yangon during December 1993. Height and weight of the children were measured and a total of 944 stool samples, including 148 non-enrolled children, were examined for the presence of intestinal parasites. Expressing the nutritional status as standard deviation scores for weight-for-height, the prevalence of wasting among 5-10 years non-enrolled school-age children was 19.8%, while that for school children of similar age was 13.9%. In addition, non-enrolled school-age children had higher infection rates than school children regarding *Ascaris lumbricoides* (66.9% vs 50.1%), *Trichuris trichiura* (38.5% vs 23.9%), and *Giardia lamblia* (5.4% vs 2.7%). The policy implication of this study is that health and nutritional status of non-enrolled school-age children needs to be promoted and this should be partly solved by the provision of regular and periodic mass chemotherapy against major intestinal parasitoses influencing nutritional status.

INTRODUCTION

The prevalence of malnutrition in school-age children is about 40% in rural Myanmar in terms of height for age or weight for age [1]. On the other hand, the prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* in school children may reach over 80%, that of *Giardia lamblia* over 21% in Yangon [2]. Malnutrition has been found to be partly contributed by *A. lumbricoides* [3-5], *T. trichiura* [6] and *G. lamblia* [7]. A survey carried out in 1990 found that over 38% of children of 5-9 years old were never enrolled in

school, and that only 1 out of 10 children enrolled in kindergarten completed primary school in 5 years, ending with a high level of dropouts [8]. There has been no comparative studies on the prevalence of malnutrition and major nutrition-related intestinal parasitoses between school children and non-enrolled school-age children in Myanmar. Therefore, this study was conducted in December 1993 in Yangon.

STUDY SUBJECTS AND METHODS

Six and seven primary schools from Tharkayta and Mingaladon townships

with a total of 3325 school children and 164 non-enrolled school-age children from neighbouring wards were included in the study. Height up to 0.1 cm and weight up to 0.1 kg of each child (1800 school children and 103 non-enrolled school-age children from Tharkayta and 1525 and 61 children respectively from Mingaladon) were measured using standard methods. Stool samples of 485 first standard students and 96 non-enrolled school-age children from Tharkayta and 311 and 52 children respectively from Mingaladon were examined for intestinal parasites with direct faecal smear method. National Centre for Health Statistics (NCHS) growth charts were used for grading children according to height for age, weight for age and weight for height (WHO, 1983).

RESULTS

Nutritional status

Among the school children in Tharkayta and Mingaladon, the males were higher in height than the females from 5 to 12 years, and the difference in height was statistically significant by single year age group from 6 to 8 years (Table 1).

Similarly, the males were higher in weight than the females from 5 to 10 years and the difference in weight was statistically significant by single year from 6-9 years (Table 1).

Among the non-enrolled school-age children in Tharkayta and Mingaladon, no definite patterns were observed either in height or weight between the males and the females (Table 2).

In comparing the mean height or weight among the 5 to 10 years old children, the enrolled school-age children were slightly taller or heavier than the non-enrolled and the differences were not statistically significant.

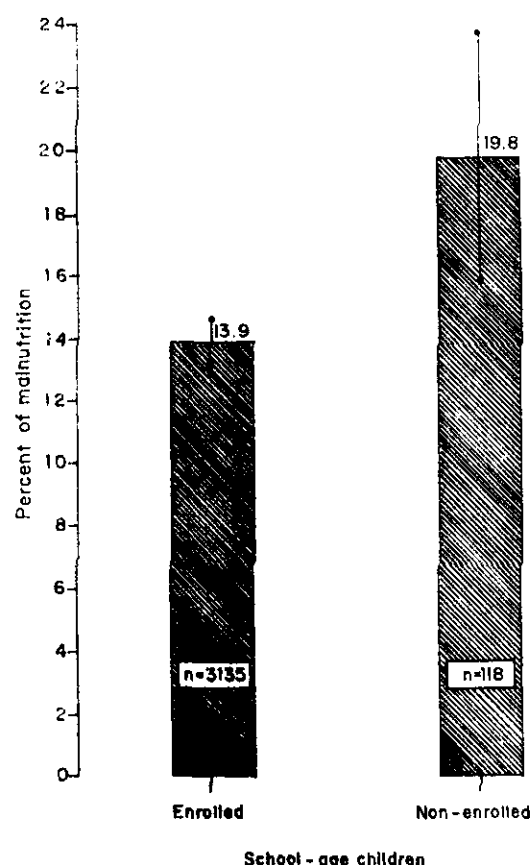


Fig. 1. Prevalence of malnutrition among enrolled and non-enrolled school-age children, 5-10 years old

In comparing the prevalence of malnutrition (moderate and severe), there was a significant difference between enrolled and non-enrolled school-age children among the 5-10 years age group in terms of weight for height (13.9 vs 19.8%, $p < 0.05$) (Figure 1), but not in terms of height for age or weight for age.

Intestinal parasitoses

In Tharkayta, the overall prevalence of *A. lumbricoides*, *T. trichiura* and *G. lamblia* in schools were 62.3% (range being 46.8 to 81.3%), 36.2% (16.2 to 47.9%) and 5.0% (1.4 to 8.8%) respectively (Table 3). The respective figures for Mingaladon were 37.7% (range being 25.8 to 44.1%), 10.2% (6.5 to 20.5%) and 2.2%

Table 1. Nutritional status of primary school children in Tharkayta and Mingaladon by sex and age, December 1993

Age in years	Tharkayta				Mingaladon			
	Male		Female		Male		Female	
	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
5	103.6±4.6 (161)	15.3±1.8 (161)	103.0±4.5 (153)	15.2±1.6 (153)	102.6±4.1 (90)	14.9±1.8 (90)	102.2±4.7 (120)	14.6±1.9 (120)
6	108.6±5.5 (242)	16.5±2.1 (242)	107.6±5.3 (200)	16.3±2.1 (200)	107.1±5.2 (169)	16.3±2.1 (169)	105.4±5.1 (144)	15.4±2.1 (144)
7	113.7±5.5 (255)	18.3±2.4 (255)	113.3±5.7 (211)	17.7±2.3 (211)	112.9±6.6 (156)	17.9±2.6 (156)	110.9±5.2 (182)	17.5±1.9 (182)
8	115.9±5.9 (127)	18.9±2.2 (127)	115.6±6.2 (105)	18.4±2.2 (105)	116.5±5.9 (168)	19.5±2.7 (168)	113.8±5.7 (143)	18.2±2.6 (143)
9	120.8±5.9 (83)	20.6±2.8 (83)	120.5±7.2 (76)	20.4±3.0 (76)	119.9±5.4 (106)	20.9±2.7 (106)	118.5±6.9 (59)	19.8±3.1 (59)
10	123.3±7.2 (51)	22.0±3.3 (51)	121.9±8.3 (40)	21.2±3.1 (40)	121.3±6.4 (60)	21.6±2.9 (60)	119.5±7.7 (34)	20.5±3.5 (34)
11	126.9±6.4 (31)	23.6±3.7 (31)	128.3±5.8 (33)	24.1±3.9 (33)	124.6±7.1 (37)	22.7±3.7 (37)	122.1±8.2 (17)	23.2±4.2 (17)
12	131.0±3.9 (10)	24.6±2.6 (10)	134.0±6.6 (9)	26.7±3.0 (9)	132.4±6.8 (18)	25.3±3.7 (18)	128.6±10.1 (11)	26.4±5.6 (11)
>13	135.8±8.5 (9)	27.5±4.5 (9)	130.5±10.1 (4)	27.3±5.9 (4)	134.3±13.0 (8)	27.3±6.7 (8)	131.4±6.7 (3)	25.6±1.2 (3)

Number of subjects in parentheses

Table 2. Nutritional status of non-school children in Tharkayta and Mingaladon by sex and age, December 1993

Age in years	Tharkayta				Mingaladon			
	Male		Female		Male		Female	
	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
5	97.7±5.3 (9)	13.5±2.0 (9)	100.0±5.7 (9)	13.9±1.6 (9)	101.0±5.1 (9)	14.3±1.6 (9)	101.5±6.4 (7)	16.2±3.7 (7)
6	104.2±4.9 (5)	14.7±0.8 (5)	103.8±3.9 (5)	15.1±1.9 (5)	113.0±6.3 (7)	15.5±1.1 (7)	108.9±5.9 (3)	16.6±2.8 (3)
7	111.5±5.6 (6)	17.5±1.8 (6)	112.5±7.3 (6)	17.3±3.2 (6)	118.9±0.0 (1)	17.2±0.0 (1)	115.6±4.9 (2)	19.2±4.2 (2)
8	115.1±5.4 (7)	19.8±1.6 (7)	112.6±6.7 (7)	17.2±2.2 (7)	113.2±8.8 (4)	21.7±1.6 (4)	111.7±9.6 (2)	18.8±0.1 (3)
9	118.5±6.5 (6)	19.9±2.1 (6)	113.8±3.6 (4)	16.7±0.3 (4)	0.0±0.0 (0)	0.0±0.0 (0)	119.7±8.5 (3)	23.5±6.2 (3)
10	124.2±5.4 (5)	21.7±2.4 (5)	124.2±9.4 (4)	22.3±6.2 (4)	122.6±9.5 (4)	19.7±2.6 (4)	110.6±2.2 (1)	16.0±1.4 (2)
11	121.4±11.1 (7)	21.9±5.9 (4)	129.3±5.7 (12)	23.8±3.7 (12)	134.4±11.1 (4)	22.7±0.2 (2)	133.1±1.8 (1)	24.2±5.5 (3)
12	125.0±6.5 (4)	20.4±2.2 (4)	126.2±11.7 (10)	22.3±4.3 (10)	130.5±0.0 (1)	25.0±0.0 (1)	131.8±6.1 (8)	21.1±2.1 (8)

Number of subjects in parentheses

Table 3. Prevalence of major intestinal parasites in Tharkayta and Mingaladon by school, December 1993

State primary school	Tharkayta				State primary school	Mingaladon			
	n	Al	Tt	Gl		n	Al	Tt	Gl
No. 5	57	61.4	29.8	8.8	No. 4	65	40.0	9.2	0.0
No. 14	140	66.4	39.3	1.4	No. 13	44	40.9	20.5	0.0
No. 25	48	81.3	47.9	8.3	No. 26	31	25.8	6.5	6.5
No. 26	68	51.5	16.2	5.9	No. 27	50	32.0	10.0	0.0
No. 28	79	46.8	24.1	3.8	No. 29	30	30.0	10.0	6.7
No. 32	87	49.4	33.3	3.4	No. 31	21	28.6	9.5	4.8
					No. 35	68	44.1	10.3	4.4
Total	479	58.9	32.1	4.4	Total	309	36.6	11.0	2.6

n = No. of primary school children tested for stool examination

Al = A. lumbricoides; Tt = T. trichiura; Gl = G. lamblia

Table 4. Prevalence of major intestinal parasites in primary school in Tharkayta and Mingaladon by age, December 1993

Age in years	Tharkayta				Mingaladon			
	n	Al	Tt	Gl	n	Al	Tt	Gl
6	237	58.2	35.4	4.6	106	44.3	9.4	0.9
7	120	57.5	30.8	4.2	91	30.8	7.7	4.4
8	53	67.9	32.1	3.8	64	42.2	20.3	4.7
9	33	54.5	27.3	6.1	22	13.6	4.5	0.0
10	19	57.9	26.3	5.3	18	38.9	16.7	0.0
11	10	60.0	0.0	0.0	6	16.7	0.0	0.0
12	2	50.0	0.0	0.0	1	0.0	0.0	0.0
>13	5	60.0	40.0	0.0	1	0.0	0.0	0.0
Total	479	58.9	32.1	4.4	309	36.6	11.0	2.6

N = No. of primary school children tested for stool examination; Al = A. lumbricoides; Tt = T. trichiura; Gl = G. lamblia

(0-6.7%) (Table 3). The age specific prevalence of the intestinal parasite was higher in Tharkayta than in Mingaladon (Table 4).

In Tharkayta, the overall prevalence of A. lumbricoides, T. trichiura and G. lamblia among non-enrolled school-age children (n = 96) were, 79.2, 56.2 and 8.3% respectively. The respective figures for Mingaladon (n = 52) were 44.2, 5.8 and 0%.

DISCUSSION

Like in the national level, the males among the school children in Tharkayta and Mingaladon are higher than the females in height or weight especially in the 5 to 10 year age group.

The finding of no definite patterns among the non-enrolled school-age children either in height or weight by age may be due to the small study size.

The greater in height or weight among school children than among children not in school reflects better nutritional status in the former group.

This is observed more clearly by comparing the prevalence of malnutrition in the two types of children in terms of weight for height, indicating that body wasting is much more among the latter than among the former.

This may be due to harbouring of higher infection rates of malnutrition causing intestinal parasites like A. lumbricoides, T. trichiura and G. lamblia among the non-enrolled children than the enrolled ones.

Children (both enrolled and non-enrolled) in Mingaladon are relatively free of intestinal parasites than those in Tharkayta. This may probably be due to better environmental sanitation and/or lower population density in the former than in the latter township.

CONCLUSION

The nutritional status and health of the non-enrolled school-age children needs to be promoted and should not be neglected. This study has policy implication in that school-age children not in school should be regularly dewormed for the major intestinal parasites just like children in schools.

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Geographical variation of biological properties of Russell's viper venom: Bago Division

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Biological properties of Russell's viper venom from four localities (Tharyarwaddy, Lepadán, Waw and Daíke-U) of Bago Division were studied according to WHO recommended methods. SDS-PAGE electrophoresis of venoms were also performed. Biological properties of venom of young adults (61-90 cm) and adults (91-120 cm) of each locality were comparable except venom of young adult vipers of Tharyarwaddy possessed weak coagulant activity and that of adult vipers of Waw has potent haemorrhagic and necrotic activities. The adult viper venom of Tharyarwaddy was two times more potent in defibrinogenating and three times in capillary permeability activities than venom of adult vipers of Lepadán. SDS-PAGE electrophoresis of venoms shows quantitative and qualitative differences in protein bands.

INTRODUCTION

Geographical variation of biological properties of venom including Russell's viper of Myanmar [1,2,3,4,5] has been reported. Snake bite is endemic in six rice growing divisions of Myanmar and biological properties of venom from Ayeyarwaddy, Yangon and Magway had been studied [6,7,8]. Understanding of the variation in biological properties and composition of venom to be used as an immunogen plays an important role in preparation of antivenom to be used for treating snake bite cases throughout Myanmar. This report concerns the study of biological properties of venom from Bago Division.

MATERIALS AND METHODS

Snakes collected from four localities of Bago Division (Tharyarwaddy, Lepadán, Daíke-U and Waw) (Fig. 1) were milked individually. Venoms were lyophilised and pooled according



Fig. 1. A map showing four localities of Bago Division where venoms were available for study

Lp = Lepadán, Th = Tharyarwaddy, Du = Daíke-U and W = Waw.

to locality and length of the snake into two groups: adult (>90 cm) and young adult (<90 cm). Venom of young adult snake of Daíke-U was not available for study.

Biological properties of venom such as haemorrhagic, necrotic, coagulant, defibrinogenating, lethality and capillary permeability increasing activities were studied according to WHO recommended technique [9]. SDS-PAGE electrophoresis of venom was also carried out. Details of the methods have already been described [5].

RESULTS

The results of the biological properties of venom from four localities are shown in the Table. Adult viper venom of Waw possessed potent haemorrhagic, necrotic and coagulant activities whereas that of Tharyarwaddy had potent defibrinogenating and capillary permeability increasing activities. Young adult viper venom of Tharyarwaddy possessed weak coagulant but potent necrotic defibrinogenating and capillary permeability increasing activities. SDS-PAGE electrophoresis of venom from different localities are shown in Fig. (2). There were qualitative and quantitative differences in protein bands.

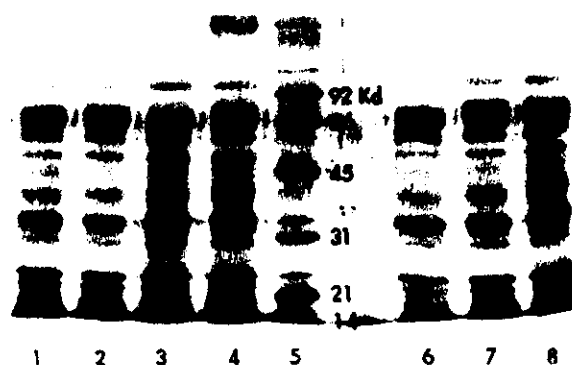


Fig. 2. SDS-PAGE electrophoresis of venom of adult and young adult vipers of Bago Division.

Lane 1 to 4 = venoms of adult vipers of Tharyarwaddy, Lepadan, Waw and Daike-U respectively. Lane 5 = molecular weight markers. Lane 6 to 8 = venoms of young adult vipers of Tharyarwaddy, Lepadan and Waw respectively

DISCUSSION

This is the 4th of division-wise study of geographical variation of Russell's viper venom of Myanmar. Studies of biological properties of venom from Ayeyarwaddy, Yangon and Magway Divisions [6,7,8] revealed that there was intra and inter divisional variation

Table 1. Biological properties of Russell's viper venoms of Bago Division

Source of venom	Length (cm)	No. of snake pooled	LD ₅₀ app. ug/mouse $\pm$ 1SD	MCD (ug)	MHD (ug/rat)	MND (ug/rat)	MDD (ug/mouse)	MCPID (ug)
Tharyarwaddy	61-90	14	4.55 $\pm$ 1.80	1.148	42.2	44.7	2.4	0.0007
	91-120	14	4.67 $\pm$ 2.00	0.251	42.2	39.8	2.4	0.0007
Lapadan	61-90	14	5.01 $\pm$ 1.67	0.195	32.4	46.8	5.1	0.0020
	91-120	10	3.76 $\pm$ 1.74	0.302	37.2	55.6	5.1	0.0028
Waw	61-90	9	3.96 $\pm$ 1.90	0.182	63.1	58.8	3.3	0.0011
	91-120	13	5.76 $\pm$ 2.40	0.141	38.5	31.6	4.2	0.0034
Daike-U	91-110	12	3.24 $\pm$ 1.40	0.436	42.7	50.1	4.2	0.0080

MHD = Minimum haemorrhagic dose

MCD = Minimum coagulant dose

MND = Minimum necrotic dose

MDD = Minimum defibrinogenating dose

MCPID = Minimum capillary permeability increasing dose

in biological properties of venom. The same inference could be drawn from this study. It is interesting to note that even from locality of closed proximity, Tharyarwaddy and Lepadon showed variation in biological properties of venom. Previous study on biological properties of venom [5] suggested that venom from Daike-U Township possessed potent lethality, haemorrhagic, necrotic, coagulant and defibrinogenating activity; however, in the present study venom of Daike-U Township has potent lethality only. Such variation in property of venom may be attributed to either seasonal or batch to batch variation of venom. It is likely that weak coagulant property of venom of young adult viper of Tharyarwaddy may be responsible for slow onset of incoagulable blood seen in patients bitten by young snakes in Tharyarwaddy Township. These findings suggest that a widely pooled venom should be used and variation in clinical features following Russell's viper bite in Bago Division is expected.

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Comparison of in vivo anti-inflammatory and antiplaque properties of
Scoparia dulcis Linn. (Dan-ta-thuka) extract and chlorhexidine
mouthwashes on normal and gingivitis subjects

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Scoparia dulcis Linn. an indigenous Myanmar medicinal plant, which is being used as mouthwashes for the management of gingival troubles by the local people. In vivo study of the 1% Scoparia dulcis Linn. watery extract mouthwash on gingival inflammation and dental plaque accumulation study in comparison with standard 0.02% chlorhexidine mouthwash on normal and gingivitis subjects. Its antiplaque activity is similar to that of 0.02% chlorhexidine.

INTRODUCTION

Dental caries and periodontal diseases are the commonest oral diseases found in the worldwide population. There are many studies into the prevention and management of these diseases. In most of the developing countries including Myanmar, periodontal problem is tending to increase due to insufficient community preventive programmes. In Myanmar, 80% of school children had gingivitis and 18% of them had periodontal destruction [1]. Baseline oral health survey in Myanmar (1990) showed 22.6% of 12 to 13 years old children were free from gum disease and only 2.6% of 65 years and above old people were free from periodontal disease.

For the management of gingivitis, local conservative therapy, i.e. removal of hard and soft deposits on the tooth surfaces, topical use of antiseptic solutions and correct use of tooth brushing methods are employed [2]. Thus a variety of antiseptic mouthwashes are seen in modern dental literatures. Almost all of these chemical agents have antiplaque property. As dental plaque is the

known initiator of gingival inflammation [3], 0.02% chlorhexidine gluconate mouthwash is widely used as a standard antiplaque agent for its surfactant action and less harmful effects [4,5]. However, the availability and the use of chlorhexidine mouthwash is not common in Myanmar.

In Myanmar, a certain number of traditional remedies or folk medicaments is used as mouthwashes or topical applicants for the management of gingival troubles [2]. Most of these folk medicaments are plant extracts and are being widely used in the rural population and also in certain percentage of urban people. Among them, a handful amount of Dan-ta-thuka (Scoparia dulcis Linn.) herbs boiled in three tea cups of water down to one-third of its initial volume i.e. decoction is commonly known to be used by local people for the management of inflamed gingiva.

Dan-ta-thuka is an herb of Scrophulariaceae family and grows wild together with grass in the whole Myanmar. It is an erect, much branched herb and is flowering and fruiting throughout the year. Flowers

are small and greenish-white colour and fruits are small capsules which dehisce when mature.

Dan-ta-thuka is reported to contain scoparic acid(s) as a major chemical component [6] and scopadulcic acid B and Scopadulin [7]. Moreover, a benzoylated diterpene alcohol named scopadulciol is present in the plants collected from Taiwan, China, Thailand and Indonesia [8].

In *vivo* and *in vitro* studies of scopadulcic acid B are reported to have anti-herpes simplex virus type 1 (HSV-1) activities [6]. In Brazilian folk medicine *Scoparia dulcis* Linn. is used for fever, pain relief, control of diabetes, management of menstrual disorders and respiratory infection. In Myanmar Dan-ta-thuka was mentioned in various use including the treatment of gum trouble [9].

The exploration and promotion of traditional lifestyles conducive to oral health was suggested by Dr. Nakajima, Director General of WHO in his 1994 World Health Day message. This message encourages us to initiate the relevant investigation of *in vivo* study of the *Scoparia dulcis* Linn. Watery extract is used in gingival inflammation and dental plaque accumulation study in comparison with standard 0.02% chlorhexidine mouthwash on normal and gingivitis subjects.

Objectives

1. To compare the amount of dental plaque accumulation using *Scoparia dulcis* Linn. watery extract and 0.02% chlorhexidine gluconate mouthwashes on normal subjects.
2. To compare the amount of dental plaque accumulation and inflammatory condition of gingiva using watery extract of *Scoparia dulcis* Linn. and 0.02% chlorhexidine gluconate mouthwashes on gingivi-

tis subjects.

MATERIALS AND METHODS

Plant materials

Scoparia dulcis Linn. was collected in Yangon area from June through September, 1994. Botanical identification was made at the Research Division, Department of Traditional Medicine.

Preparation of 1% *Scoparia dulcis* Linn. watery extract mouthwash

The plant was cleansed with tap water. The aerial parts of the fresh plant were cut into small pieces. 1.25 Kg of cut pieces was boiled with deionized water (0.5 L/0.1 Kg) for four hours continuously. The decoction was filtered and filtrate was concentrated on a boiling water bath until semisolid extract was obtained. The semisolid extract was weighed and the moisture content was determined. Total solid extract was found to be 4.68g/100g fresh weight. It was serially diluted to 1% solution for mouthwash (Solution A).

Preparation of 0.02% chlorhexidine gluconate mouthwash

2.0 ml of 5% chlorhexidine gluconate stock solution (chlorhexidine gluconate solution Generico medical practice, Almere-Holland) was serially diluted with 500 ml of deionized water to get 0.02% working solution (Solution B).

Study design

In this study double-blind and cross-over clinical trial design was employed.

Selection of subjects

60 male students of first year Dental Medicine from Lanmadaw Hostel are screened and selected as 10 normal and 14 gingivitis subjects. Gingivitis

subjects are randomly selected as group I for 7 and group II for 7 students. 80 female students of first year Nursing Science were screened and selected as 15 normal and 16 gingivitis subjects. Gingivitis subjects are randomly divided into two equal groups.

Selection criteria

The normal subjects must be between 18 to 21 years old and there should be no signs of apparent gingival inflammation on inspection. For gingivitis subjects, there should be signs of gingival inflammation at a group of teeth in each sextant. All subjects must have full complement of teeth present in their mouth. There is no excessive carious activity in their teeth. All subjects should have regular use of tooth brushing with tooth brush and toothpaste. There is no sign of acute infections in the mouth and students having any dental treatment and have had any dental treatment within 3 months are excluded. The students with any known systemic diseases, including chronic diseases and infectious diseases are also excluded.

Consent

The selected subjects are briefly explained about the clinical trial, its objectives and procedure and about the drugs used in this experiment. The print consent form is distributed among the volunteer subjects for this clinical studies (Appendix I).

First recording

An experienced periodontist was assigned for all recordings. First recording was done at 3 to 5 pm on the first day. Examination was done on a chair using natural light. A standard plane mouth mirror (Ash. # 10) and a CPITN (E) Probe was used in all the recordings. These instruments are boiled for 20 minutes and

6% Aseptol solution was used as cold sterilization between each recording. Using the assessment form (Appendix II), gingival colour, contour, consistency, surface texture and position are recorded. Gingival Inflammatory Index was scored at each sextant and CPITN scoring was done accordingly. The subjects were allowed to chew one disclosing tablet for half minute and rinse once with plain water. Then the mean plaque score was recorded in each sextant.

After initial recording, normal subjects (25) were requested to stop brushing for 1 week. They were allowed to use the given mouthwash (A or B) according to the given instruction.

Instruction for mouthwashing

20 ml (approximately) of solution (using the marking on the bottle) was allowed to hold in the mouth for one minute and rinse with plain water for 3 times after that.

Mouthrinsing was done one time before breakfast and one time before going to bed i.e. 2 times a day. After each rinsing, the subjects recorded their rinsing on a given form (Appendix III). Gingivitis subjects were allowed to use their regular oral hygiene practices without any modifications. They were allowed to use Solution A or B separately.

Second recording

Second recording was done between 3 to 5 pm on the 7th day after one week.

Rest period

After 2nd recording the experiment was stopped for 3 weeks to avoid the residual effect of drugs in the oral cavity.

Cross-over experiment

On the 5th week, another recording

was done and the subjects were instructed to use the different mouthwash solution with same procedure. The final recording was made on the 6th week of experiment.

Dropped out

In normal group one male student and 2 female students are dropped out due to some reasons. In gingivitis group I and II, one male and one female student dropped out due to unavoidable circumstances.

RESULTS

The accumulation of dental plaque on normal subjects: the amount of dental plaque accumulation and CPITN scores on 22 normal subjects (males = 9, females = 13) before and after using mouthwashes are compared in Table 1a and b.

Table 1a. Amount of plaque score and CPITN scores on 22 normal subjects using 1% Scoparia dulcis Linn. watery extract mouthwash

	Plaque score	CPITN score				
		0	1	2	3	4
1st Record	0.89±0.13	4.2	1.2	0.6	-	-
1 wk after	1.10±0.24	4.0	1.4	0.6	-	-

't' test = $P > 0.1$ N.S.

There is no significant difference in plaque score and CPITN scores using 1% Scoparia dulcis Linn. watery extract mouthwash.

Table 1b. Plaque score and CPITN using 0.02% chlorhexidine gluconate

	Plaque score	CPITN score				
		0	1	2	3	4
1st Record	1.1*±0.21	4.8**	0.7**	0.5	-	-
1 wk after	0.9*±0.16	4.4**	1.1**	0.5	-	-

't' test = * $P > 0.1$ N.S.

= ** $P < 0.05$ Significant

Normal subjects using 0.02% chlorhexidine mouthwash showed no significant difference in plaque score, however the mean number of sextant scoring (0) (i.e. normal) is significantly reduced and the mean number of sextant scoring (1) i.e. (Gingival bleeding) is significantly increased.

Gingivitis subjects were randomly selected in two groups, i.e. each group contained 6 males and 7 females. Their inflammatory index, plaque score and CPITN scores are compared. The results of gingivitis group I are shown in Table 2a and b.

Table 2a. Gingival inflammatory index, plaque score and CPITN scores of gingivitis group I using 1% Scoparia dulcis Linn. watery extract mouthwash

	Inflam: index	Plaque $\bar{x}$	CPITN score				
			0	1	2	3	4
1st Record	0.4*	1.2*	1.2*	1.1*	3.7*	-	-
1 wk after	0.7*	0.9*	1.2*	1.1*	3.7*	-	-

't' test = $P > 0.1$ N.S.

There is no significant difference in inflammatory index, plaque score and CPITN scores of gingivitis group I using 1% Scoparia dulcis Linn. watery extract mouthwash for a week.

Table 2b. Gingival inflammatory index, plaque score and CPITN scores of gingivitis group I using 0.02% chlorhexidine mouthwash

	Inflam: index	Plaque $\bar{x}$	CPITN score				
			0	1	3	3	4
1st Record	0.6*	1.1*	0.4*	2.1*	3.5*	-	-
1 wk after	0.4	1.1	0.5	2.0	3.5	-	-

't' test = $P > 0.1$ N.S.

The gingival inflammatory index, plaque index and mean number of sextant of CPITN scores are not significantly different in gingivitis patients using 0.02% chlorhexidine mouthwash for a week.

The results of gingivitis group 2 are shown in Table 3a and b.

Table 3a. Gingival inflammatory index, plaque index and CPITN scores of gingivitis group 2 using 1% Scoparia dulcis Linn. watery extract mouthwash

		CPITN score				
Inflam:	Plaque	0	1	2	3	4
index x	score x					
1st Record	0.4*	1.2*	2.1*	0.9*	3.0*	-
1 wk after	0.7*	0.9*	2.6*	0.4	3.0	-

't' test = $P < 0.05$ significant

Gingivitis group 2 using 1% Scoparia dulcis Linn. watery extract for a week shows significant increase in inflammatory condition. However, the amount of plaque score was significantly reduced. The mean number of normal sextants was significantly increased and the mean number of bleeding sextants was significantly reduced.

Table 3b. Gingival inflammatory index, plaque score index and CPITN scores of gingivitis group 2 using 0.02% chlorhexidine mouthwash

		CPITN score				
Inflam:	Plaque	0	1	2	3	4
index x	score x					
1st Record	0.15*	1.1**	2.5	0.6	2.9	-
1 wk after	0.28	1.2	2.5	0.6	2.9	-

't' test = ** $P < 0.1$ N.S.

* $P > 0.05$ significant

The inflammatory index of gingivitis group 2 using chlorhexidine mouthwash was significantly increased. However, there is no significant difference in plaque score and CPITN scores.

DISCUSSION

The results of these experiments show some points to be discussed. In normal subjects, accumulation of dental plaque was controlled by the use of mouthwashes without routine oral hygiene practices. The normal subjects were requested to refrain from tooth brushing after first recording for one week. There should be increase in accumulation of plaque due to lack of tooth brushing. However, both mouthwashes have certain antiplaque property and thus the mean plaque scores and CPITN score are not different. It is of interest to note that Scoparia dulcis Linn. has some antiplaque property compare to standard 0.02% chlorhexidine mouthwash.

Gingivitis subjects (group 2) showed slight increase in gingival inflammation after using 1% Scoparia dulcis Linn. mouthwash, but the plaque score was not increased. This can be possible in some cases. The inflammation of gingiva is not always related proportionally with the amount of dental plaque accumulated on the tooth surface [3]. It depends on the maturity of dental plaque. Matured dental plaque can produce bacterial toxins and enzymes which can provoke gingival inflammation [2]. In order to find out the maturity of dental plaque, microbiological study is an essential requirement. Most of these results in this experiment show no significant difference in most of the scores, i.e. antidental plaque activity of the test solution is no less than that of 0.02% chlorhexidine standard.

During this experiment only one normal subject complained that the

sensation of a notable astringency in his mouth persisted. However, there is no other signs of complications reported. It is of interest to discuss about the difference of two agents in comparing their organoleptic characters. Scoparia dulcis Linn. mouthwash is the herbal extract and thus have slightly sweet smell and the solution is slight turbid whereas chlorhexidine is free of such properties.

The investigation reveals some comparable results in between Scoparia dulcis Linn. watery extract and chlorhexidine mouthwashes. However, these findings stimulated further studies for chemical analysis, antibacterial properties and long-term usage of Scoparia dulcis Linn. extracts.

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SHORT REPORT

**Family planning: Focus Group Discussion (FGD) among married women
in Phaunggyi Township, Myanmar**

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Family planning and birth spacing need serious national consideration in Myanmar as borne out by indicators of static maternal mortality rates in hospitals and unwanted pregnancies leading to septic abortions which comprised half of maternal deaths [1]. In order to solve this problem, it is required to develop appropriate measures like provision of education on contraception, making available the necessary requirements and methods for avoiding unwanted pregnancies. For proper health education and counselling, the emphasis on assessment of the level of knowledge, attitudes and practices of married women on contraceptions should be made before launching a nationwide scale family planning programme. Thus an attempt has been made to identify some aspects on KAP of contraception using Focus Group Discussion method (FGD) [2] in a group of married women from a location in Phaungyi Township, 40 miles north of Yangon, Myanmar. A total of 41 married women were recruited for participation in the study. The youngest participant was 24 years and the oldest was 47 years. The educational status of women varied from illiterate to University/High School graduates and most of them were school drop-outs from various grades.

Focus group discussions were carried out with 6-9 participants facilitated by a member of the study team. The group was encouraged to talk

freely and spontaneously on ten topics concerning knowledge about contraception, attitudes towards family planning and methods practised for birth spacing. One study member recorded the outcomes of discussions. Six sessions were carried out and each session lasted for an hour with a range of 40 to 60 minutes.

All the respondents agreed that family planning is important, the main reasons being improvement of maternal and child health and the economic status of the family. One 39 year old woman said "We can not work with little baby as we have to support our family as much as we can" and one multip who has five children said "If you have baby one year after another, you may get sick, I mean mother can not take care of herself or the baby well". It represented the group discussion as family planning is a key issue for maternal and child health. Knowledge on various methods was one of the main topics and it showed that most of the respondents knew different methods of contraception, only 2 recently wedded respondents could not give any comment. Oral contraceptive pills and contraceptive injections were the most commonly used contraceptives, the main reasons being easy accessibility and cheapness. They also believed that oral contraceptive pills and contraceptive injections were the safest methods. Only few were familiar with the rhythm method, very few used condoms or

intrauterine devices. Some respondents also had experience with traditional methods such as massage on uterus and traditional medicines. Although some women had plans for sterilization for medical conditions such as varicose veins, many of the respondents used contraceptive methods as advised by their close friends and neighbours. They seemed to be shy to mention rhythm and condoms. It was evident that they were sensitive to talk about condom. One or two women could mention what condom was and these were educated persons. The majority were in need of family planning, they acknowledge the fact that they would like an interval of at least 2 years between subsequent pregnancies and some stated that they did not want more than 2 or 3 children. However, some stated that they would produce as many babies as God decides for them. All educated respondents did not want more than 3 children. The respondents stated that they want counselling on contraception and that a family planning clinic should be opened in their location.

Regarding the role of the husband in contraception, most respondents said their husbands accepted their use of

contraceptive methods. Most husbands were reluctant to undergo male sterilization thinking that it might affect their health.

It is evident from this study that family planning is important, the main reasons being improvement of maternal and child health and economic status of the family. Family planning and birth spacing services should also include adequate information on contraceptive methods other than oral contraceptive pills and contraceptive injections. Accessibility to other contraceptive methods should also be initiated.

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SHORT REPORT

Does liver involve in Russell's viper bite patients with systemic envenomation?

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Envenomation by Russell's viper is known to cause severe local tissue reaction and coagulopathy [1]. Local effects at the bite site, haemorrhagic manifestations in the kidney, spleen, liver and peritoneum, intestinal inflammation and glomerular changes with acute tubular necrosis have been well documented following snake bite. Different degenerative changes in vital organs including liver have also been studied in envenomed animals [2]. Most of these toxic manifestations are due to the presence of hydrolytic enzymes in snake venoms. A study on the distribution of ^{125}I -labelled Russell's viper venom (RVV) in mice showed that liver was found to be the 4th organ of maximal distribution of venom [3]. Jeyarajah of Sri Lanka [4] reported raised serum levels of bilirubin and alanine aminotransferase in some of his patients with Russell's viper bite although liver damage was not manifested clinically and hepatic pathology was not mentioned in postmortem examination performed on a few of his patients who died of other complications. De-Silver [5] also reported a case of Russell's viper bite patient who became comatose with evidence of acute hepatocellular failure and died on 3 days after bite showing severe fatty changes with hepatocellular necrosis at postmortem examination.

Thus, it prompted us to investigate hepatic involvement in Russell's viper bite victims with systemic envenomation. Therefore, 14 known Russell's

viper bite patients admitted to Tharyawaddy Township Hospital with systemic envenomation confirmed by incoagulable blood with clinical proteinuria and positive venom antigen detected by Enzyme Linked Immunosorbent Assay (ELISA) were studied. Blood samples were collected at the time of admission and once a day for a minimum of 5 days' duration or up to the day of transfer or expired. These samples were stored at -20°C until analysed at Department of Medical Research where liver function tests (LFT) such as total bilirubin, unconjugated and conjugated bilirubin, alkaline phosphatase, serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were performed. Although 6 patients out of 14 had oliguric acute renal failure (ARF), none of these patients showed clinical features of hepatic involvement. There was also no change in LFT during the 5 days clinical course in all these patients including two victims who died of oliguric ARF within 5 days after bite. The reasons for the absence of clinical features of hepatic involvement and no LFT change in these patients may be that the patients had received antivenom therapy to neutralise the toxic venom and recovered, or died of ARF or other causes before liver damage was manifestable clinically and biochemically. Although that liver is one of the major organs of venom distribution due to its role for excretion through gastrointestinal tract, according to

our study there is no evidence of clinical involvement nor biochemical changes. The possible reasons may be that RVV have no direct or indirect hepatotoxic effect and besides, the liver cells may not be vulnerable to the damaging effect of venom. However, no attempts have been made to confirm liver involvement histologically since liver specimens could not be obtained from these victims.

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