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**Comparison of the effect of combinations of parenteral artemisinin derivatives
(I/M artemether, I/V artesunate) plus oral mefloquine with intravenous
quinine plus oral tetracycline on patients with cerebral malaria:**

**A multicentre trial
(PRELIMINARY REPORT)**

Tin Shwe, **Myint Lwin, *Tan Myint Maung, *Ko Ko Hla, *Htin Aung Saw,
****Soe Aung, *****Than Htay, ***Kyi Kyi Ngwe, *Kyin Htwe,
*Hla Min & *Nilar Win*

*North Okkalapa General Hospital & CRU (Malaria) DMR

**Director (Research) DMR

***Mawlamyine Civil Hospital

****Department of Health (VBDC)

*****PyinOoLwin Civil Hospital

In order to find out the best drug combination for treatment of cerebral malaria at less equipped hospitals, 105 cases of cerebral malaria belonging to Mawlamyine, PyinOoLwin and North Okkalapa hospitals were studied in a controlled trial of three regimens. (1) Intramuscular artemether total dose 480 mg plus mefloquine 750 mg in a single dose given through nasogastric tube at day 0. (2) Intravenous artesunate total dose 240 mg plus mefloquine 750 mg as in regimen 1. (3) Intravenous quinine dihydrochloride 600 mg in 180 ml infusion of dextrose saline given over 4 hours. The dose is repeated every 8 hours until the patient can swallow the tablets. Then oral quinine sulphate tablets were given 600 mg 8 hourly. Total period of quinine therapy is 7 days. Tetracycline 250 mg capsules were given 6 hourly for 7 days (started via nasogastric tube while the patient is unconscious). There was no significant difference in overall mortality rate, mean parasite clearance time, mean fever clearance time and mean time to regain consciousness between the three groups. Thus quinine-tetracycline (if necessary to supplement with artemether-mefloquine at 48 hours if the patient failed to respond to initial treatment) is suggested, as the drug of first choice for the management of cerebral malaria in Myanmar.

INTRODUCTION

Promising results of artemisinin derivatives together with mefloquine on patients with cerebral malaria have been reported from China and Myanmar [1-6]. But a controlled study comparing the efficacy of combinations of artemisinin derivatives plus mefloquine with standard drug quinine and tetracycline in the treatment of cerebral malaria has not been con-

ducted on civilian population. Hence this study is carried out.

Objective

To find out the best drug combination for the treatment of cerebral malaria in hospitals with limited facilities.

EXPERIMENTAL DESIGN AND METHODS

It is a controlled multi-center drug

trial, conducted during May 1993 to October 1994. Patients with unrousable coma with or without other complications and acute *P. falciparum* infection admitted to Mawlamyine, PhinOoLwin, and North Okkalapa General hospitals were chosen for the study. Duration of coma in the patients needed to be for at least 30 minutes in those with generalized convulsions. Associated complications due to malaria were properly recorded.

Exclusion criteria

Patients aged above 65 years, children under 12 years, pregnant women, patients who refused to give consent and patients with renal impairment (urine output less than 400 cc in 24 hours), patients with other encephalopathies due to bacterial meningitis and locally prevalent viruses and patients with other known diseases such as tuberculosis (as judged from the history and clinical examination of the patients) were excluded from the study.

Subjects allocation

The patients were treated with the undermentioned 3 groups of drugs in succession.

a. Artemether and mefloquine

Intramuscular injection artemether 160 mg initially and 80 mg 12 hourly for another 4 doses. Mefloquine (MLQ, Mepha) 750 mg was given via nasogastric tube on day 0.

b. Artesunate and mefloquine

Intravenous injection artesunate 60 mg for each injection given at 0, 4, 24 and 48 hours. MLQ 750 mg was given via nasogastric tube on day 0.

(Artemether and artesunate injections prepared from GULIN No. 2 Pharmaceutical Factory, Guangxi, China were used).

c. Quinine and tetracycline

Quinine 10 mg/kg initially followed by 10 mg/kg 3 times a day for 7 days. Quinine dihydrochloride was given by IV infusion with dextrose saline taking 4 hours for each dose. Quinine sulphate was given orally when the patient became conscious and could swallow. Dose of tetracycline was 250 mg QID for 7 days. Quinine and tetracycline manufactured from Myanmar Pharmaceutical Factory were used for the trial.

Patients already on prior antimalarial therapy were included in the study. The drug that had been used on the patients during the last 24 hours before admission to hospital were recorded.

Criteria for discontinuation

Treatment failure was designated when (1) Parasite load did not fall to more than 25% of initial level after 48 hours of drug treatment or if (2) Clinical and laboratory result became worse at 48 hours after admission to the hospital.

All treatment failure cases were treated with the alternative drug regimen artemether-mefloquine for patients who failed to respond to quinine-tetracycline and quinine-tetracycline for those who failed to respond to artemether-mefloquine or artesunate-mefloquine.

All patients were admitted to hospital for a minimum of 7 days. Routine laboratory and biochemical examinations were done as follows:

- (1) Blood for CP, PCV and glucose level was determined daily on 15 patients in each group.
- (2) ECG on days 0, 3 and 7 was done on 15 patients belonging to each group.
- (3) Other biochemical tests were done whenever required.

Body temperature was recorded every 6 hours until it had been normal for 24 hours and 12 hourly thereafter.

Thick and thin blood films were taken every 12 hours until no asexual forms of *P. falciparum* were seen in the peripheral blood for 24 hours and thereafter daily till day 7 and on days 14, 21 and 28.

Chi-square and Student's 't' test were used for statistical analysis.

RESULTS

From the four centers, 105 consecutive patients were collected for evaluation. 15 sets of patients were from North Okkalapa Hospital and 10 sets of patients each were from Mawlamyine and PyinOoLwin hospitals. Their base line characteristics and associated complications are shown in Table 1.

Among the patients treated with artemether-mefloquine 3 (8.5%) needed to change the drug at 48 hours. Among the patients treated with artesunate-mefloquine, 6 (17.1%) patients needed to change the drug at 48 hours. Among the patients treated with quinine-tetracycline, 5 (14.2%) patients needed to change the drug at 48 hours.

Among the patients treated with artemether-mefloquine 7 (20.0%) expired. Among the patients treated with artesunate-mefloquine 10 (28.6%) expired. Among the patients treated with quinine-tetracycline 5 (14.3%) expired. All patients who changed over the drug regimens survived.

There was no significant difference in the overall mortality rate of the three groups of patients.

The patients who died within 24 hours of arrival at hospital were excluded from the study. The mortality rates of the 3 groups were 11.4%, 11.4% and 5.7% respectively. There was also no significant difference

among the three groups of drugs.

There was no significant difference in the mean parasite clearance time, mean fever clearance time and mean time to regain consciousness in the 3 groups of drugs studied (Table 2 and 3).

Due to local reasons it was difficult to record the name of the drugs with which the patients had been treated before they were admitted to hospitals.

There was not a single patient with severe toxic signs and symptoms that can be related to the drugs. Among the 15 patients who had taken ECG at days 0, 3 and 7 no changes in ECG were recorded. Patients with changes in neurological signs and symptoms were also not seen.

DISCUSSION

In this study there was no significant difference in the overall mortality rates as well as in the mortality rates of patients who died after 24 hours of arrival at the hospital, in all 3 groups of patients studied. This is different from the previous report where Kyaw Win *et al.* in 1992 [2] based on their studies in the military population found that the combination of intramuscular artemether-tetracycline or intravenous artesunate-mefloquine is superior to quinine-tetracycline in reducing the mortality rate in severe cases of human cerebral malaria. They reported overall mortalities in the 3 groups of patients as 14% in patients treated with artemether-mefloquine, 8.3% in patients treated with artesunate-mefloquine and 34.3% in patients treated with quinine-tetracycline.

The difference in the results of the two trials may be due to:

1. Lower dosage of artemether and artesunate used in this study,

Table 1. Base line characteristics and associated complications of the three treatment groups

	Regimen 1 (artemether + mefloquine) n=35	Regimen 2 (artesunate + mefloquine) n=35	Regimen 3 (quinine + tetracycline) n=35
Mean age $\pm$ SD years	40.25 $\pm$ 2.6	32.65 $\pm$ 7.6	35.62 $\pm$ 5.9
Female/male ratio	1:2.6	1:1.5	1:1.7
Mean initial parasite density $\pm$ SD (Bruce Schwatt scale)	1.51 $\pm$ 1.15	2.01 $\pm$ 0.85	1.81 $\pm$ 0.95
P.C.V.	22.5%	23.2%	20.5%
Associated complications	No. of patients		
Jaundice	2	2	2
Hyperparasitaemia	0	1	0
High temperature $>105^{\circ}\text{F}$	0	2	0
Anaemia	2	1	2

Table 2. Mortality rate of patients treated with 3 groups of drugs

Drug Group	No. of patients studied	Overall mortality	Mortality rate within 24 hours of arrival at hospital	Patients who need change of drug at 48 hrs
Artemether + mefloquine	35	7(20.0%)*	4(11.4%)**	3(8.5%***)
Artesunate + mefloquine	35	10(28.6%)*	4(11.4%)**	6(17.1%***)
Quinine + tetracycline	35	5(14.3%)*	2(5.7%)**	5(14.2%***)

* Differences not significant $p < 0.05$ ** Differences not significant $p < 0.05$ *** Differences not significant $p < 0.05$

Table 3. Mean parasite clearance time, mean fever clearance time and mean time to regain consciousness in three groups of drugs studied

Drug group	No. of patients studied	Mean parasite clearance time (hrs) $\pm$ SD	Mean fever clearance time (hrs) $\pm$ SD	Mean time to regain consciousness (hrs) $\pm$ SD
Artemether + Mefloquine	35	51.4 $\pm$ 33.1*	65.0 $\pm$ 33.6**	42.7 $\pm$ 29.3***
Artesunate + Mefloquine	35	42.6 $\pm$ 21.6*	69.4 $\pm$ 35.6**	40.1 $\pm$ 35.2***
Quinine + Tetracycline	35	60.1 $\pm$ 37.1*	62.5 $\pm$ 36.3**	57.0 $\pm$ 40.0***

* Differences not significant $p < 0.05$ ** Differences not significant $p < 0.05$ *** Differences not significant $p < 0.05$

where total dosage of artemether was 480 mg and that of artesunate was 240 mg. In the study reported by Kyaw Win *et al.*, 1992 [2], the dosage of artemether was 600 mg and artesunate was 300 mg. In this study, dosage of mefloquine was 750 mg whereas in the previous report, the dosage of mefloquine was 1000 mg.

2. The differences might also be due to the differences in the population studied.

In this study, all of the patients were civilians who had been treated with some form of unidentified antimalarial injection before they were admitted to the hospitals.

The majority of the patients in the previous study were on chemoprophylaxis and only those not responding to antimalarials may have developed cerebral malaria.

3. The source of the patients

In this study, most of the patients contracted malaria from areas close to the hospitals and calculation was made from the pooled data of the three hospitals. In the previous study, the majority of the patients were brought to Mingaladon from Kayin, Kaya and Shan States along Thai-Myanmar border where multidrug-resistant strains of *P. falciparum* are well known.

The previous study carried out by our group in Thayawady hospital also showed the mortality rate of cerebral malaria patients treated with quinine alone was around 20% [4]. The higher mortality rate of quinine-tetracycline treated cerebral malaria population (34%) in other study might be due to variation in quinine sensitivity of the two groups of patients. Thai-Myanmar border had higher degree of mefloquine resistance than the other parts of Myanmar. Since

mefloquine is structurally related to quinine, these patients may not be responding well to quinine.

The lower mortality rate recorded in this study may also be due to the timely changing of drug regimens. The patients were supplemented with the alternative combination regimen, if the parasite level did not fall satisfactorily at 48 hours or if the clinical conditions deteriorated.

In this country, quinine has been used for nearly a hundred years. Although resistance to quinine was suspected in Myanmar since 1916 [7] true resistance to quinine is rare. So far, our unit has recorded only one case with R2/R3 resistance to quinine [8]. The patient was from Kawthoung Township, Thai-Myanmar border. We feel that the rate of development of resistance to quinine is very slow. Artemisinin compounds however have high recrudescence rates between 25-40% in areas where the drug has never been used. It is very likely that drug resistance to artemisinin compounds would develop in the very near future.

Hence quinine-tetracycline (supplemented with artemether-mefloquine at 48 hours if the patient failed to respond to initial treatment) is suggested as the drug regimen of first choice for the management of cerebral malaria patients among the general population of Myanmar.

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The authors wish to thank Medical Superintendent of Mawlamyine General Hospital, Director of Health, Department of Health, Mon State, Malariologist, Mandalay VBDC and Medical Superintendent of North Okkalapa General Hospital for all their help to carry out this project. This study was done with the grant from WHO/TDR ID 920379.

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Seasonal pattern of enterotoxigenic Escherichia coli (ETEC) in children
under three years of age

*Mar Mar Nyein, *Tin Aye, **Khin Maung U, **Myo Khin, *Phyu Phyu Win
& **Thane Toe

*Bacteriology Research Division

**Clinical Research Division
Department of Medical Research

Children aged one month to three years from Yangon Children Hospital were studied. A total of 923 cases of diarrhoea and 932 cases of control were included in this study. The total isolation rate of ETEC was 232 cases (25.2 percent) and 55 cases (5.9 percent) from diarrhoea and control cases respectively. In the diarrhoea group, out of 232 cases of ETEC, 43 cases (4.7 percent) were STLI, 134 cases (14.5 percent) were SI and 55 cases (6.0 percent) were LI. In the control group, out of 55 cases of ETEC, 6 cases (0.6 percent) were STLI, 21 cases (2.3 percent) were SI and 28 cases (3.0 percent) were LI. Although ETEC was found in children all the year round, the incidence rate was higher during the months of February to July with the highest peak of 47.0 percent in June. ETEC occurred in the control cases with the maximum isolation rate of 16 percent in June.

INTRODUCTION

Enterotoxigenic Escherichia coli (ETEC) is one of the most common causal agents of acute diarrhoea among poor hygienic tropical countries [1-3]. Acute diarrhoea with malnutrition still causes high mortality of children under five years old in this country. For the effective control of diarrhoeal disease one needs to know the major causative agents and how they are distributed among children in relation to different seasons. The purpose of this study is (a) to describe the seasonality of ETEC isolation in Yangon Children Hospital and (b) to find out the relationship between ETEC and serotypes so as to make easier in identification of ETEC without using sophisticated equipment.

MATERIALS AND METHODS

The study period started from Febr-

uary 1982 and ended in January 1984.

In the year 1982, 376 diarrhoea cases and 389 control cases were studied and in the year 1983, 547 diarrhoea cases and 543 control cases were studied.

The stool samples were collected as reported by Huilan et al. [4]. Three isolated colonies from MacConkey agar having the morphological appearance of E. coli were confirmed by biochemical tests and stored in nutrient agar stabs and processed for enterotoxin assays. E. coli were grown in Trypticase Soy Broth (BBL) in the shaking incubator (280 rpm/min) at 37°C for 14-16 hours and the supernatant was used for enterotoxin assays. Gentamycin was also supplemented. Heat-stable (ST) enterotoxin assay was done by using suckling mouse [5] and heat-labile enterotoxin (LT) assay was done by using Chinese Hamster Ovary cells

[6] and by modified BIKEN test [7]. Statistical analysis was done by using Student's 't' test. Serotyping was done according to the method of Ewing [8].

Most of the ETEC strains were serotyped by Dr. B. Rowe, Division of Enteric Pathogens, Central Public Health Laboratory, London, UK.

RESULTS

In figure 1, the distribution of Enterotoxigenic *E. coli* (ETEC) isolated

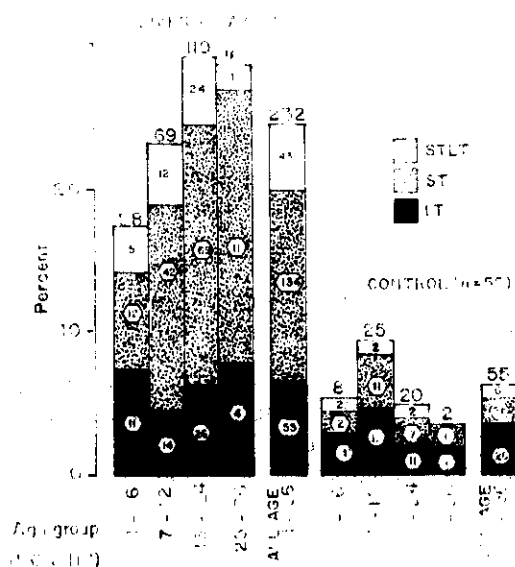


Fig. 1. ETEC distribution in diarrhoea and control children (YCH)

from children under three years of age in Yangon Children Hospital during two successive years in 1982 and 1983 is shown. A total of 923 cases of diarrhoea and 932 cases of controls were included in this study. ETEC was isolated from 232 cases (25.2 percent) of diarrhoea and 55 cases (5.9 percent) of control and it was found to be significant ($p < 0.001$) between the two groups studied. Out of 232 cases of ETEC detected

in the diarrhoea group, 43 cases (4.7 percent) were STLT, 134 cases (14.5 percent) were ST and 55 cases (6.0 percent) were LT. In the control group, out of 55 cases of ETEC, 6 cases (0.6 percent) were STLT, 21 cases (2.3 percent) were ST and 28 cases (3.0 percent) were LT. The isolation ratio of ETEC was not related to age group, but it seem less in younger age group than in 6-month age group. Annual incidence of ETEC in 1982 and 1983 is shown in figure 2. The highest frequency of ETEC was from February to July (hot wet season). The highest isolation rate of ETEC (47 percent) was found in June 1982.

Relationships between Enterotoxigenic *Escherichia coli* and serogroups from diarrhoea and control case of children are shown in Table 1. It was found that 0126 was more prevalent than other serogroups. Out of 278 cases the isolation rate of 0126 was found to be 21.9 percent (61 cases) and that of 0114 was 7.9 percent (22 cases) and they were associated well with the ST and LT type of *E. coli* respectively. Thirteen number of 0128ab were also isolated and they were associated well with the STLT group. However, 79 isolates of ETEC did not related to any of the serogroups tested and thus claimed as untypable serogroups.

'O' serogroups with their specific 'H' antigens are shown in table 2. It was found that in 0114 serogroup 14 cases were found to be nonmotile, 7 cases showed H49 and 1 case showed a rough type. Also in 0126 serogroup 1 case showed H7, 55 cases showed H12; one case showed H30 and 4 cases showed untypable 'H' serotype. In 0128ab it was found that one case each showed H8 and H18 and 11 cases showed H12.

From the unknown type of 'O' serogroups 'H' serotypes were scattered ranging from H1 to H13.

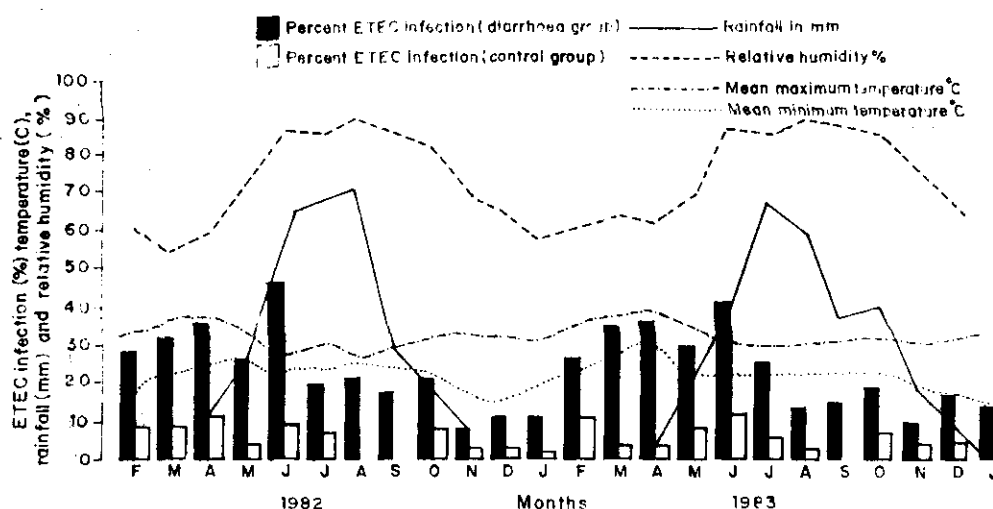


Fig. 2. Seasonal pattern of ETEC in acute diarrhoea and control children from Yangon Children Hospital

DISCUSSION

ETEC is undoubtedly an important cause of diarrhoea in all age groups in areas of poor hygiene, particularly in the tropics. Various investigators from Arizona, Ethiopia, South Africa, Taiwan, Mexico, Philippine, Pakistan, India and Bangladesh reported that the isolation rate ranges from 11 to 35 percent [9-19]. However, in a study in Bangladesh, ETEC was isolated from only one of 39 sources of drinking water [20]. In a study reported from Canada [21] ETEC was suggested to be important. In a Swedish study in 1977 [22] and a small survey in Britain [23] ETEC was found to be uncommon as a cause of indigenous sporadic diarrhoea. In the United States of America, 2,000 staff of a national park and visitors developed diarrhoea caused by a strain of *E. coli* 06 which produces ST and LT [24].

In this study also the isolation of 25.1 percent and 5.9 percent of ETEC from diarrhoea cases and con-

trols respectively showed that ETEC played an important role as one of the causative agents responsible for acute diarrhoea in children. It was also indicated that ETEC infection was not age specific. Lacero et al. [15] also reported that ETEC isolation rate showed a definite seasonal pattern with a peak of 40 percent in June in Philippine. This is in accordance with the present study where the highest isolation rate (47 percent) was obtained in June. It was also noted that ETEC was present throughout the year ranging from 9 to 47 percent in the diarrhoea group and 2 to 13 percent in the control group. Thus, ETEC plays an important role in diarrhoea as a causative agent as well as a common organism among children without diarrhoea. Thus, the source and route of infection were to be thoroughly well understood to prevent diarrhoea.

The first outbreak of infantile enteritis due to ETEC which belonged to 0159 serogroups and produced heat-stable toxin was reported in 1976

Table 1. Relationship between enterotoxigenic *Escherichia coli* and serogroups from diarrhoea and control cases of children from Yangon Children Hospital in 1982-1983

Serogroups '0'	ST		LT		STLT		Total		Grand total
	D	C	D	C	D	C	D	C	
6	2	1	7		2		11	1	12
7			2	1	1		3	1	4
8	4		2	1	4		10	1	11
9a	2		1				3	0	3
17			1		2	1	3	1	4
20		2		1	8		8	3	11
21	1			1			1	1	2
25			1		4		5	0	5
27	6	1					6	1	7
39	1						1	0	1
44	1				1		2	0	2
60					1		1	0	1
64	1				1		2	0	2
73	1						1	0	1
78	1				3		4	0	4
86	3			1			3	1	4
88			1				1	0	1
106			1				1	0	1
113			2				2	0	2
114			16	6			16	6	22
115	1						1	0	1
117			2				2	0	2
126	58	1	1		1		60	1	61
128ab					13		13	0	13
139		1					0	1	1
146			1				1	0	1
148	3	1					3	1	4
153	2			1			2	1	3
159			2		1	1	3	1	4
166					1		1	0	1
167			2				2	0	2
169	1						1	0	1
R	<	2	1	1	1	0	2	3	5
O ?	42	7	19	6	4	1	65	14	79
Total	120	16	62	19	48	3	240	38	278

ST = *E. coli* producing heat-stable toxin

LT = *E. coli* producing heat-labile toxin

STLT = *E. coli* producing both heat-stable and heat-labile toxin

D = Diarrhoea

C = Control

[25]. In the same year epidemic outbreak occurred in United States of America which belonged to 078 and produced only ST [26]. Further outbreak in Britain was also effected

by *Escherichia coli*, an O6 strain that produced both ST and LT. An outbreak in Britain affected 10 of 18 babies in the hospital in Gloucester and was caused by a strain of *E.*

Table 2. 'H' serotypes in accordance with 'O' serogroups

'O'	Tested	H	No. of cases	'O'	Tested	H	No. of cases	'O'	Tested	H	No. of cases
6	12	H	1	78	4	H12	2	167	2	H5	2
			11			H18	1	169	1	H-	1
7	4	H-	1			H20	1	R	5	H10	1
		H18	2	86	4	H2	1			H21	1
		H23	1			H11	1			H25	2
8	11	H-	2			H18	2			H52	1
		H9	5	88	1	H25	1	0 ?	79	H-	12
		H12	1	106	1	H45	1			H1	1
		H15	1	113	2	H7	1			H5	4
		H21	2			H10	1			H6	1
9a	2	H-	3	114	22	H-	14			H7	3
17	4	H18	3			H49	7			H8	1
		H51	1			H(R)	1			H9	1
20	11	H-	9	115	1	H5	1			H10	4
		H12	1	117	2	H27	2			H12	13
		H25	1	126	61	H7	1			H14	1
21	2	H-	1			H12	55			H16	2
		H12	1			H30	1			H18	3
25	5	H	1			H?	4			H19	5
		H42	4	128AB	13	H8	1			H20	1
27	7	H-	1			H12	11			H21	2
		H7	1			H18	1			H25	1
		H20	5	139	1	H19	1			H26	6
39	1	H28	1	146	1	H19	1			H27	4
44	2	H1	1	148	4	H28	4			H29	2
		H27	1	153	3	H12	3			H32	1
60	1	H18	1	159	4	H25	1			H33	1
64	2	H-	1			H34	1			H35	2
		H45	1			H?	2			H38	1
73	1	H1	1	166	1	H15	1			H?	7

E. coli 06 that produced ST and LT. The source and route of infection in those outbreaks were uncertain [27]. Rowe and his colleagues first demonstrated a relationship between *E. coli* and travellers' diarrhoea in 1970 and serotyping proved evidence of 0148 and only later it was discovered that the strain produced ST [28]. Merson also reported that ETEC was found to be responsible for 45 percent of the cases of traveller's diarrhoea in Mexico [29] while Gorbach indicated 72 percent of those with diarrhoea were excreting ETEC [30]. In the study 0126 was found to be

related to ST only and 0114 to LT-producing *E. coli*. 0128ab was also associated with STLT type of *E. coli*. Moreover, 0148 showed a relationship with ST group.

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Anthelmintic property of Myanmar pineapple (Nanat) on experimental models

Aye Than, Yi Mon Myint, S.J. Tha & Chit Maung

Pharmacology Research Division
Department of Medical Research

The anthelmintic property of Myanmar pineapple (*Ananas sativa* L.) juice was investigated by using both *in vitro* and *in vivo* test models on *Ascaris suum*. In the *in vitro* test model, the pineapple juice, at a concentration of 40-80 mg/ml was found to produce immobilization of the worms leading to death within a 2 day period. In the *in vivo* test model using pigs, the pineapple fruit, doses as low as 10 g ingested food were found to purge *Ascaris suum* within 16 hrs of ingestion. The lethal effect was assumed to be due to cuticle digestive effect of bromelain, which is present in an average of 0.29 mg percent in pineapple.

INTRODUCTION

In Myanmar, ascariasis can be considered as a significant health problem especially in childhood age group. On a regional basis, it was reported that there is a high prevalence of human intestinal ascariasis in Myanmar [1]. Various workers had reported that the intestinal helminthiasis particularly *Ascaris lumbricoides* was endemic in wet and humid rural areas of Myanmar [2]. Reviews concerning the national experiences of ascariasis control measures and programmes have also been made by various authors [3-5] since *A. lumbricoides* was the principle intestinal helminth and its infection rate was found to be as high as 77 percent in the population as a whole. Even though many anthelmintics are available to combat ascariasis, many people subsist on the belief that locally available edible fruit, if consumed from time to time could control round worm infestation [6]. The suggestive anthelmintic activity of the unripe pineapple fruit had also been reported previously by a western worker [7]. Low cost and availability in abundance of the fruit throughout the year make pineapple

a good anthelmintic agent which can be enjoyed both as food and at the same time used to control ascariasis and this justifies the need for investigation for its reputed action.

MATERIALS AND METHODS

Preparation of the fresh fruit juice

The fruits used for the experiment were purchased from the markets of Yangon area. The fresh fruits were weighed, sliced into small pieces and homogenized in a homogenizer (Polytron Homogenizer Kinematica, Switzerland). The homogenized material was squeezed through cheese cloth to remove fabric materials. The expressed fruit juice was partitioned into solid and liquid layer by means of centrifuge (IEC International Centrifuge, International Equipment Company, USA). Then the turbid supernatant was decanted and pooled in a 500 ml conical flask.

Extraction of crude bromelain

Crude bromelain from local pineapple fruits was extracted using the method of Bonner & Varner [8]. The small

piece of fresh fruit were ground gently in mortar and pestle and strained through cheese-cloth. The filtrate obtained was cooled to 0-4°C. Then it was centrifuged at 2000 rpm for 10 minutes and was decanted. The supernatant obtained was cooled and equal volume of acetone was added. Then it was centrifuged at 2000 rpm for 10 minutes again followed by decantation. The precipitate obtained was dried under reduced pressure and was powdered in mortar and pestle so as to obtain the enzyme powder. Such process was repeatedly done about 2-3 times until no more precipitate formed. The bromelain content was measured against authentic casein by the method of Kunitz [9].

Determination of anthelmintic activity of fresh fruit juice on the in vitro test model

The anthelmintic effect of fresh fruit juice on in vitro test model was determined using the method of Sen and Hawkins [10]. The test was carried out by using the various concentrations of ripe fruit juice in Tyrode's solution (8 ml/50 ml, 16 ml/50 ml and 32 ml/50 ml), which were equivalent to 20, 40 and 80 mg/ml in residual form, concurrently with piperazine hexahydrate in Tyrode's solution for various final concentration levels (4, 8 and 16 mM/ml). For the in vitro screening, the worms were collected from the pig intestines at post-mortem at the slaughter house as stated by Aye Than et al. [11,12]. Ten alive and fresh worms were used for each concentration level of treatment. Observations on the motility of each worm were noted regularly every 24 hours for a duration of 4 days, with the daily renewal of the bathing solution. The results were compiled and presented graphically.

The anthelmintic activity of the above fruit juice was again tested on

in vitro test model using the method of Goodwin [13] as modified by Aye Than et al. [11]. The dose of the test agent employed was 40 mg/ml (pH 5.5-6.5) bath concentration in Tyrode's solution. Both the frequency or rate of contraction and magnitude or height of contraction of the worm were continuously monitored for a maximum of 24 hours on a direct writing ink recorder physiograph, E & M). Control test was concurrently performed employing only the Tyrode's solution. The results were compiled and analysed statistically.

Determination of anthelmintic effect of bromelain on the in vitro test model

The in vitro test was repeatedly carried out by using the test substances like bromelain extract 40, 80 and 160 mg/ml final bath concentration and those of fresh juice 8, 16 and 32 ml in 50 ml each of Tyrode's solution. Also included were the concurrent controls containing only the vehicles. The effects of the two agents are compared and illustrated graphically.

In vivo study of the anthelmintic activity of A. sativa on swine ascariasis

A total of 25 pigs, of either sex, weighing 20 ± 0.6 kg were used for the investigation. The presence of worm's eggs in the stools of the pigs was primarily studied. Each animal with ascaris infection was kept in isolation. Edible portion of the fruit was sliced into small pieces and fed to the pigs, before the morning feed for three consecutive days. Each animal received a randomized one tested dose level of A. sativa, (0, 5, 10, 20 and 40 g/kg) and the number of parasites purged daily was observed and recorded for 3 consecutive days. The results were analysed statistically.

RESULTS

Anthelmintic activity of fresh fruit juice on the in vitro test model

The lowest dose (4 mM) of piperazine immobilized the worms in 12 hrs, the intermediate dose (8 mM) in 6 hrs and the highest dose (16 mM) in 3 hrs. At the dose range used, the response was found to be dose-dependent. In the concurrently performed experiment using *A. sativa* juice, its high dose (80 mg/ml bath concentration) caused the worm to death in 11.5 hrs; and low dose (40 mg/ml bath concentration) in 16 hrs (Fig. 1). Plotting on the log-dose basis

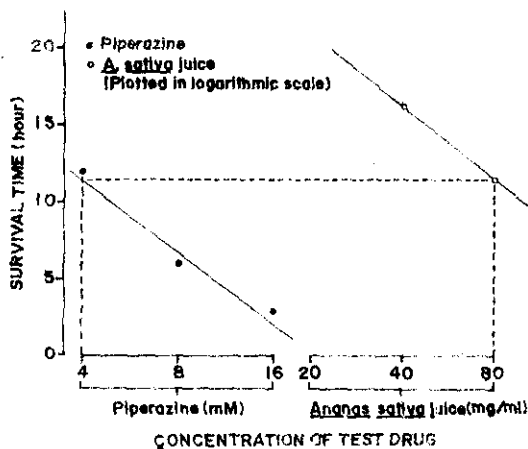


Fig. 1. Comparison of *in vitro* anthelmintic activity between *A. sativa* juice and piperazine on *A. suum*

with respective equivalent dose interval against the response measured in terms of survival rate, there was found to be a good parallelism. Thus it was able to compare the relative potency, which showed that the commonly employed dose of piperazine (4 mM) was equipotent to 80 mg/ml of *A. sativa* juice in the aspect of immobilizing the worm.

The time-course of the effect of the

juice on the frequency or rate of contraction and magnitude or height of contraction of the parasite after various time intervals is shown in Fig. 2. There was no stimulation of

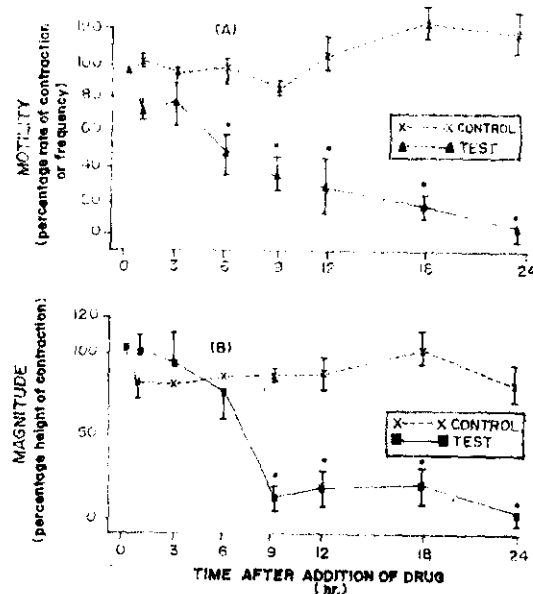


Fig. 2. Time-course of the effect of *A. sativa* fruit juice on the motility (a) or percentage of rate of contraction and magnitude (b) or percentage of height of contraction of *Ascaris suum*

Each point represents the mean of at least thirteen observations, and vertical bars indicate standard errors of the means. Difference in levels of statistical significance between pre- and post-values are denoted as * = $p < 0.001$.

the parasite activity resulting in both the increase in frequency or in the heightening of the magnitude when compared with that of the control. However, motility of the worm reduced later and resulting in paralysis with significant immobilization in 9 hrs for both the frequency ($p < 0.005$) and magnitude ($p < 0.025$), after which the test parasites never

recovered and died.

Anthelmintic activity of extracted bromelain on the in vitro test model

Anthelmintic effect of extracted bromelain and fresh juice on the in vitro test model is shown in Fig. 3.

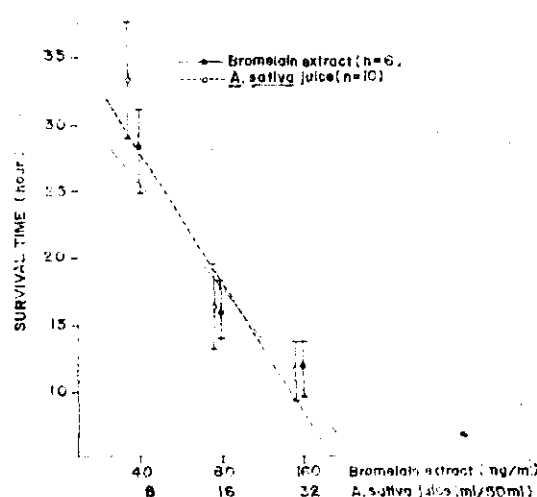


Fig. 3. Comparison of the anthelmintic effect of bromelain and juice on the in vitro experiment model of A. suum. Vertical bars indicate standard errors of the means.

Even though there was slight reduction in the anthelmintic activity of the juice on an equipotent basis, there was no significant difference between them when treated statistically.

Anthelmintic activity of fruit on in vivo test model

The results of in vivo test are shown in Table 1. The control animal did not pass any worms during the 3 day experimental period. In terms of worms purged per animal, the anthel-

minthic activity of the 10 mg/kg, 20 mg/kg and 40 mg/kg were 1.2 ± 0.58 , 4.0 ± 2.55 and 3.2 ± 1.32 respectively. Therefore, fresh A. sativa fruit had shown a certain anthelmintic activity at the low dose range of 10 g/kg body weight and further showed obvious activity at the highest dose level of 20 to 40 g/kg body weight, even though there was no marked significant difference between the two higher doses employed, which meant the 20 g/kg attained the maximum. None of the worms passed were motile.

DISCUSSION

It was observed that the juice of ripe pineapple showed an anthelmintic activity on the in vitro test models. A concentration as low as 40 mg/ml was able to kill the parasite by general disintegration of the cuticle. The juice paralysed the worm to death, without any intervening stimulatory phase on the motility of the worm. It was also reported that the death of the worm had been reputed to be caused by the unripe juice of pineapple [7]. The anthelmintic mechanism of action of the fruit was therefore deduced to be direct cuticle digestion effect on the worms. The process was suggested to be carried out by the fruit enzyme bromelain (40-160 mg/ml) as had been shown by the present work. The bromelain content of Myanmar pineapple was 0.29 mg% which was not significantly different from that of the species from other parts of the world [7,8].

A further investigation on the in vivo test model using swine had confirmed the anthelmintic action of the ripe fruits of pineapple (20 g/kg). A confirmatory observation of passing dead worm was also made in the in vivo experiments. Sixty percent of the test swine purged 1-3 worms

Table 1. In vivo oral anthelmintic effect of fresh A. sativa fruit on ascaris-infested swine

Dose (g/kg)	No. of observations	Response	
		Percent of swine dewormed	Worm purged per animal (Mean $\pm$ S.E.) (range)
0	5	-	-
5	5	-	-
10	5	60	1.2 $\pm$ 0.58 1 - 3
20	5	80	4.0 $\pm$ 2.55 1 - 14
40	5	80	3.2 $\pm$ 1.32 2 - 8

when fresh fruit 10 g/kg was administered orally. Eighty percent of the swine responded to a dosage of 20 g/kg, purging 1-14 worms. The highest dosage of 40 g/kg was effective on 80% of the test swine, purging 2-8 worms.

It was also reported that the clinical trial on the anthelmintic action of local pineapple fruit was also investigated on a total of 228 Myanmar children. Single daily oral administration was administered for two consecutive days of either the test-fruit, 6 g/kg or the placebo. The fruit was found to be effective in a significant number of the worm infested children. Moreover, its anthelmintic action became more and more pronounced with the degree of infestation in the individual children [14].

In conclusion, it is deduced that the anthelmintic dosage should be approximately a medium size (approx. 200 g) fruit for the adult, half for the youngster and one quarter for the children. It is suggested that 3-day repeated administration of the fresh ripe fruit is rather to be advocated than a single-dose therapy to achieve satisfactory deworming results.

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Investigation of antibacterial activity of three traditional medicine formulations

*Mar Mar Nyein, **Chit Maung & ** Mya Bwin

*Bacteriology Research Division

**Pharmacology Research Division
Department of Medical Research

Three Traditional Medicine Formulations which are widely used by local people were investigated for antibacterial activity using 14 species of bacteria. The bacteria include one specie each of Escherichia coli, Klebsiella pneumoniae, Streptococcus pyogenes and Vibrio cholerae; two species each of Proteus, Salmonella and Staphylococcus and 4 species of Shigellae. The formulations were Ah-bein-nyin, Heleikdasonna and Nandwin-nganzay which contain herbs and chemicals and have been used as antipyretic or in the treatment of urinary disorders, gastrointestinal disorders and cardiovascular disorders. Fifty per cent alcoholic extract of these drugs were found to possess some antibacterial activity on certain bacteria. Moreover, extracts from 7 plants namely, Saxifraga ligulata (Wall) (Nat-hsay-gamone), Capparis sepiaria (Sugaut-net), Holoptelea integrifolia (Pyauk-seik), Zizyphus oenoplia (Baung-bet), Hygrophila spinosa (Su-padaung), Mitragyna parviflora (Htain-they) and unidentified sp. (Thetyin-kadoe) were also tested on the above bacteria. It was observed that Saxifraga ligulata, Capparis sepiaria and Zizyphus oenoplia showed antibacterial activity on some bacteria.

INTRODUCTION

It has been reported that there are many antibacterial drugs including those isolated from herbs [1,2,3]. In search of new substances active against microorganisms pathogenic for man, some have been taken at random, though some have been tested as group of plants already known or suspected to possess useful medicinal properties. The beginning of intensive research in this field is marked by the work of Osborn [1] who made an enormous survey of higher plants in order to detect the presence of substances antibiotic towards Staphylococcus aureus and Escherichia coli. He studied 2,300 species of plants most of them being Angiosperms, belonging to 166 families. This paper is dealing with the search for antibacterial agents from 7 plants and 3 drugs which are quite extensively used in traditional medi-

cine.

There are no information on the use of Saxifraga ligulata (Wall) known as Nat-hsay-gamone in other countries but it has been used extensively for various ailments since it was introduced by Sayadawgyi U Ottamathara (Taungdan Sayadaw). Capparis sepiaria (Sugauk-net), Holoptelea integrifolia (Pyauk-seik), Zizyphus oenoplia (Baung-bet), Hygrophila spinosa (Su-padaung), Mitragyna parviflora (Htain-they), unidentified sp. (Thetyin-kadoe) or (Ahyaw-khan-myt) have been used in traditional medicine as various remedies. The three traditional medicine formulations known as (Abein-nyin), (Haleikdasonna ngan-hsay) and (Nandwin - ngan-hsay) have been used since ancient times as antipyretic or in the treatment of urinary disorders, gastrointestinal disorders and cardiovascular disorders.

MATERIALS AND METHODS

Plant extraction

Extraction method for *S. ligulata* (pectin free and pectin)

A 5.0 g portion of 50% ethanolic extract was suspended in 200 ml of distilled water with constant shaking in a wrist-action shaker and then centrifuged. The particle-free supernatant layer was transferred into a separating funnel and run down dropwise into four volumes of 95% ethanol, kept stirring with a magnetic stirrer. A visible precipitation occurred and the resultant mixture was kept in a fridge for 2-3 hours to ensure a complete flocculation of the pectate materials. It was then centrifuged and pectate-free supernatant layer was separated through further filtration. The clear filtrate thus obtained was concentrated under reduced pressure and low temperature. Both the pectin and pectin-free fractions were dried in desiccating chamber for subsequent activity screening.

Fifty per cent alcoholic extract

It was prepared by refluxing crushed powder of air dried parts in petroleum ether to remove chlorophyll and waxy substances. The residue after extracting with petroleum ether was then air dried and extracted with 50 per cent ethanol. It was then concentrated under reduced pressure at low temperature (38-40°C). The final concentrate thus obtained was dried in a vacuum desiccator. An appropriate concentration was prepared from this extract for subsequent activity screening experiments.

The watery extract

It was prepared as described by Mar Mar Nyein et al. [5].

Plant extracts used for antibacterial activity

- (1) *Saxifraga ligulata* (Wall.) (Nat-

hsay-gamone) (root): - 50 per cent ethanolic extract, pectin free and pectin were used for antibacterial activity.

- (2) *Capparis sepiaria* (Sugaut-net) (root bark): - 50 per cent alcoholic extract.
- (3) *Holoptelea integrifolia* (Pyauk-seik) (oil free seeds) - 50 per cent alcoholic extract.
- (4) *Zizyphus oenoplia* (Baung-bet) (stem bark): - 50 per cent alcoholic extract.
- (5) *Hygrophila spinosa* (Su-padaung) (whole plant): - 50 per cent alcoholic extract.
- (6) *Mitragyna parviflora* (Htain-thay) (stem bark): water extract.
- (7) Unidentified sp. (Thetyin-kadoe) (root): - 50 per cent alcoholic extract.

Traditional medicine formulations

- (1) Abein-nyin (အဘိညာဉ်) - 50 per cent alcoholic extract.
- (2) Haleikdasonna-nganzay) (ဟိလိကသုဉ်းငါးဆေး) - 50 per cent alcoholic extract.
- (3) Nandwin-nganzay (နန်းတွင်းငါးဆေး) - 50 per cent alcoholic extract.

Test organisms

1. *Escherichia coli* (WR 2/69)
2. *Klebsiella pneumoniae* (DMR)
3. *Proteus mirabilis* (WR 3/69)
4. *P. vulgaris* (WS 53/69)
5. *Salmonella paratyphi* B (NHL)
6. *S. typhi* (NHL)
7. *Shigella boydii* (NHL)
8. *S. flexneri* (NHL)
9. *S. shigae* (NHL)
10. *S. schmitzi* (NHL)
11. *Streptococcus pyogenes* (DMR)
12. *Staphylococcus aureus aureus* (WS 16/69).
13. *S. epidermidis* (WR 1/69)
14. *Vibrio cholerae* eltor (MPI)

Antibacterial activity of the extract

It was done as described by Mar Mar

Nyein et al. [5]. For Streptococcus pyogenes blood agar (5 per cent sheep blood) was used as a medium and serum broth was used for seeding the culture.

RESULTS

As shown in Table 1, 50 per cent alcoholic extract and pectin free substances of S. ligulata showed an antibacterial activity on E. coli, P. mirabilis, P. vulgaris, S. flexneri, S. shigae and S. aureus aureus. The pectin obtained from extraction did not show any antibacterial activity.

The 50 per cent alcoholic extracts of C. sepiaria showed the activity on E. coli, P. mirabilis, P. vulgaris, S. shigae and S. aureus. So also, H. integrifolia showed the activity on K. pneumoniae, S. typhi, S. aureus aureus and V. cholerae. Moreover, Z. oenoplia showed the activity on E. coli, P. mirabilis, P. vulgaris, S. flexneri, S. Shigae, S. aureus and V. cholerae.

The antibacterial activities of tradi-

tional medicine formulations are shown in Table 2. It was found that 50 per cent alcoholic extracts of the drugs tested were found to possess more of anti-bacterial activity. The watery extract also showed some activity and no activity was found when tested with powder alone or with residue of the extract. It was found that Nandwin-nganh say showed an antibacterial effect on Klebsiella pneumoniae, Salmonella paratyphi B, Shigella boydii, S. schmitzi, Staphylococcus aureus aureus, Streptococcus pyogenes and Vibrio cholerae. 50 per cent alcoholic extract of Abainnyin also showed antibacterial activity on E. coli, K. pneumoniae, Proteus mirabilis, Proteus vulgaris, S. paratyphi, S. boydii, S. schmitzi, S. aureus, S. pyogenes and V. cholerae. 50 per cent alcoholic extract of Haleikdasonna also showed an antibacterial activity.

DISCUSSION

Since the discovery of activity of

Table 1. Effect of S. ligulata on some bacteria

Tested organisms	Zone of inhibition (mm)					
	<u>S. ligulata</u> ¹	<u>S. ligulata</u> ²	<u>S. ligulata</u> ³	<u>C. cepiaria</u> ⁴	<u>H. integrifolia</u> ⁴	<u>Z. oenoplia</u> ⁴
<u>E. coli</u>	18	18	0	26	0	17
<u>K. pneumoniae</u>	0	0	0	0	10	0
<u>P. mirabilis</u>	14	17	0	13	0	14
<u>P. vulgaris</u>	15	17	6	11	0	14
<u>S. typhi</u>	0	0	0	0	12	0
<u>S. flexneri</u>	19	16	0	0	0	12
<u>S. shigae</u>	18	17	0	15	0	11
<u>S. aureus aureus</u>	19	19	0	15	9	9
<u>S. epidermidis</u>	0	12	0	NT	NT	NT
<u>V. cholerae</u> Eltor	0	0	0	0	11	9

disc diameter = 5 mm

1 = 50 per cent ethanolic extract

2 = pectin free

3 = pectin

4 = 50 per cent alcoholic extract

NT = Not tested

Table 2. Effect of the traditional medicine formulations on some bacteria

Tested organisms	Zone of inhibition (mm)		
	Nandwin-ngansay	Abein-nyin	Haleikdasonna*
<i>E. coli</i>	6	13	0
<i>K. pneumoniae</i>	10	12	0
<i>P. mirabilis</i>	8	10	7
<i>P. vulgaris</i>	6	10	8
<i>S. paratyphi</i> B	14	15	8
<i>S. typhi</i>	8	7	0
<i>S. boydii</i>	9	10	0
<i>S. flexneri</i>	8	8	0
<i>S. schimtzii</i>	10	11	0
<i>S. aureus aureus</i>	10	12	13
<i>S. pyogenes</i>	9	12	8
<i>V. cholerae</i> Eltor	10	12	11

disc diameter = 5 mm

*50 per cent alcoholic extract

plant origin on bacteria, the search for antibacterial activity and the constituents has been carried out extensively to date [6-11]. It was also noted that some reputed medicinal plants used extensively in the cure of bacterial diseases possessed no pronounced antibiotic activity quite contrary to expectation. Probably, additional compounds may be present in the extract which have properties antagonistic to the inhibitory substances.

In the plant *S. ligulata*, 50 per cent alcoholic extract and pectin free substances were found to be effective on most of the bacteria but when tested with the powder itself, dissolved in saline, there was no activity. It might be due to the fact that it could not diffuse through the media before it inhibits the growth of bacteria. The pectin itself from the plant also had no antibacterial activity and it was true as in pure pectin. The other three plants, *C. sepiaria*, *H. integrifolia* and *Z. oenoplia* which are also used in traditional medicine also showed the antibacterial activity. The remaining two plants *H. spinosa*

and *M. parviflora* did not show any antibacterial effect.

In this study, it was also noted that the extracts from traditional medicine formulations showed a smaller zone than from extracts of plant alone and it might be due to be effect of small amount of ingredients combined in it. However, it seems to have effective upon most of the bacteria tested by Abein-nyin and Nandwin-ngansay than in Haleikdasonna-ngansay. These three drugs are used in traditional practices as antipyretic and in treatment of urinary disorders, gastrointestinal disorders or cardiovascular disorders. Nandwin-ngansay consists of 36 ingredients, out of which 24 are of plant origin. Abein-nyin consists of 13 ingredients and out of which 6 are of plant origin. Haleikdasonna consists of 6 materials out of which 4 are of plant origin. The other ingredients are mineral, salt and animal products. It was reported that Haleikdasonna was found to have an antipyretic activity on TAB-induced pyrexial volunteers [4]. The *S. ligulata* extract was found to be most promising and it should be

further evaluated clinically.

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Knowledge, attitudes and behaviours on HIV infection/AIDS among clinical students and house surgeons in Mandalay

*Khin Ye Myint, **Chit Soe & *Than Than Kyaing

*Mandalay General Hospital

**Yangon General Hospital

Data were collected to assess the current level of knowledge, attitude towards prevention and actual practices among clinical students and house surgeons in Mandalay in 1994. It was found that there were class related differences in KAP scores of medical students. In addition, they could correctly answer the difference between AIDS and HIV infection as their education advanced ($\chi^2 = 45.55$, $p < 0.005$), but there was no difference with regard to knowledge of AIDS counselling ($p > 0.1$) and the use of condom ($p > 0.1$). Only 49.5% would like to take care of AIDS patients in their wards. To avoid treating AIDS patients is the main reason in 38.6% of them. At the same time, 23% of students believed that AIDS should be prevented by isolating HIV infected cases. Percentage of presence of risk behaviour are nearly the same for the three different classes. 27.2% of students had heterosexual and among them, 51.1% reported always using condom while 10.2% reported never using condom at all. It may be due to the misperception about the transmission or inability to break down the old socio-cultural constraints or just due to the fact that information increases knowledge but does not change behaviour. An integrated and comprehensive educational programme and periodic feedback is recommended.

INTRODUCTION

AIDS is a disease caused by a new and deadly virus HIV remaining in the body for years before any damage shows up as visible symptoms. Once it develops, in all known cases, it has always proved fatal.

WHO estimated in 1990 that over 8 million adults were infected with HIV and that a little over one-third, three million, were women [1]. HIV spreads almost entirely through identifiable behaviours and specific action. At present there is no cure for AIDS and no vaccine is available to protect against it. The key stone of prevention of HIV infection is information and health education as HIV transmission can be prevented through well-informed and responsible behaviour. AIDS epidemic has become pandemic

since the end of 1988 [2] and Myanmar is not an exception.

To provide information to those concerned in the effective development of education and training programmes for clinical students and house surgeons in Mandalay, we have undertaken a cross sectional study on knowledge, attitude and practice among them.

MATERIAL AND METHODS

A total of 310 medical students, 109 from 3rd M.B.,B.S., 99 from Final Part (II) M.B.,B.S. and 102 house surgeons (HS) from Mandalay General Hospital were studied. A questionnaire was used to collect information.

Information related to (1) knowledge: aetiology, transmission, diagnosis, risk factors, prevention and counselling

(II) attitude: HIV testing and dealing with sero-positive persons and (III) behaviours: clinical practice and risk behaviour were obtained. Questions answerable by either single response or multiple response were applied.

The duration of the study was 3 months starting from 1st June 1994. Chi-square and T-test were used for statistical significance.

RESULTS

Table 1. Total no. age & KAP scores of medical students and house surgeons from Mandalay General Hospital

	3rd M.B.		Part II		House Surgeon	
	M	F	M	F	M	F
No.	58	51	67	32	64	38
Mean age	22.95		25.74		28.36	
Mean KAP score	66.62		71.09		75.58	
S.D.	8.97		9.08		8.74	

Table 1 shows the total number, age and KAP scores of medical students and house surgeons from Mandalay General Hospital. KAP scores were increased with level of education ($P < 0.001$). A total of hundred marks were given for correct answers and KAP scores show percent of correct answers.

Figure 1 points out how they first heard about HIV/AIDS. 31% got information from newspapers and 26% from magazines. Almost all students heard about condom and counselling (99% and 91.4%).

Figure 2 presents whether they can recognise the difference between the term HIV infection and AIDS. Although 34.8% of 3rd MBBS students can recognise the difference, more than 75% of HS can do so ($P < 0.005$).

Fig. (1) HOW DID YOU FIRST HEAR ABOUT HIV / AIDS?

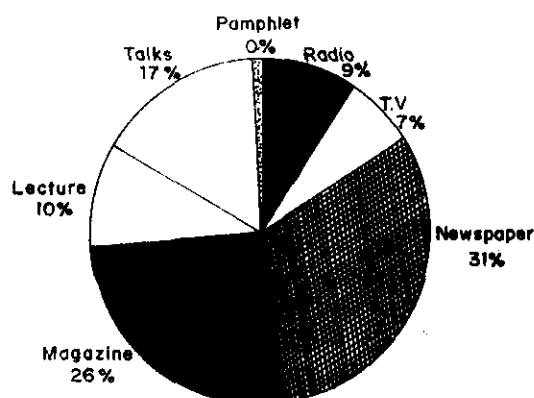


Fig. (2) CAN RECOGNIZE DIFFERENCE BETWEEN HIV INFECTION AND AIDS

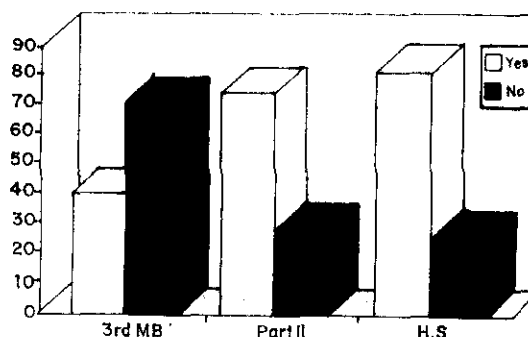


Fig. (3) DO YOU BELIEVE THAT YOU GET ENOUGH INFORMATION ABOUT AIDS?

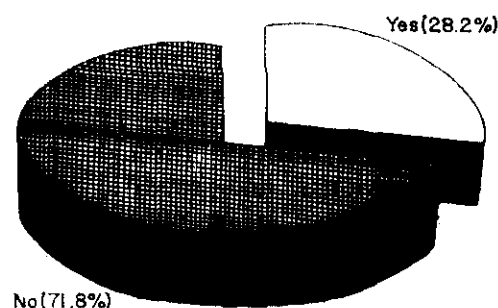


Figure 3 spotlights the fact whether they believe they got enough informa-

tion about AIDS. Unsurprisingly 71.8% said "No".

Regarding the attitude issues, 96.9% think AIDS is an important problem in this country. 94.8% believe HIV/AIDS can be prevented. However, only 49.5% would like to take care of AIDS patients in their wards. To avoid treating AIDS patients is the main reason in 38.6% of them. At the same time 23% of students believed that AIDS should be prevented by isolating HIV infected cases (figure 5). 95.5% of medical students think early HIV testing should be done for health personnel who are at high risk, but the frequency suggested is different. 19% suggested 3 monthly and 39% suggested voluntarily (figure 4). Majority of them considered

Fig. (4) HEALTH PERSONNEL SHOULD BE TESTED FOR HIV INFECTION

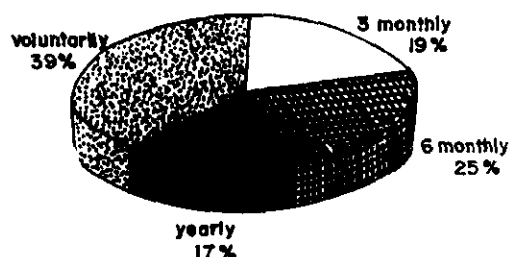
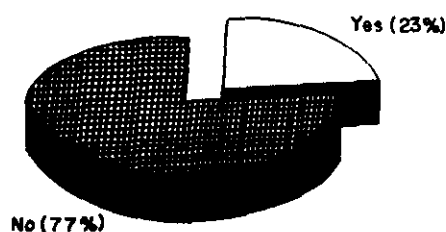


Fig. (5) WILL YOU PREVENT AIDS BY ISOLATION?



IVDA as the main route of transmission in this country. At the same time they also think there is a high risk of transmission at present from

GP (25.6%), from hospital (21.7%), from blood transfusion and hospital (42.9%) and from non-commercial sex (10.4%).

Fig. (6) PRESENCE OF RISK BEHAVIOUR IN DIFFERENT CLASSES

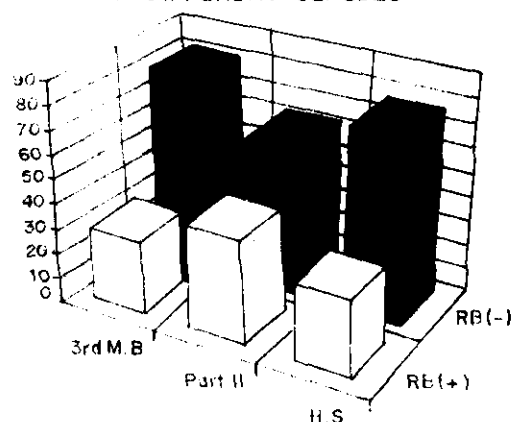


Figure 6 shows the presence of risk behaviour in different classes. Nearly 30.5% of students declare to have risk behaviour like IVDA, using sex toys, exposure to commercial sex etc. Analysis of risk behaviour shows 27.2% had heterosexual, among them, 51.1% reported they always use condom, while 28.4% use sometimes and 10.2% reported never used at all.

DISCUSSION

Our study shows that there was class related difference in KAP scores of medical students of Mandalay but there was no difference between KAP scores of students with risk behaviour and that of non-risk. Lack of combined theoretical and clinical teaching may account for the fact. This should be considered in planning and implementing AIDS prevention programme. Although their knowledge on HIV infection were found to be relatively high and their attitude towards prevention were in the neutral range, actual

preventive behaviour was low and unsafe sexual practice was high. In our study, the use of condom for extramarital sex was also traced. It was shown only 27.2% of those exposed to heterosex, 51.1% used condom regularly while 28.4% used sometimes and 10.2% never used at all. So the results suggest that current HIV prevention programme has not even penetrated into those having clinical training in hospital.

While 96.3% of students believed AIDS can be prevented with strict precaution, 50.5% would not like to take care of AIDS patients in their wards. It shows that many had misperception about non-viable routes and the risk of HIV infection among health care workers. The same thing was found in a AIDS related knowledge, attitude and practice study among a random sample of Michigan Licensed emergency medical service professionals in 1988[3-5]. 71.8% of medical students from our study thought they did not get enough information about AIDS. A study was made in Michigan in 1990 and more than 70% of respondents believed that mass media provided insufficient information on AIDS [3-6].

Those who can not recognise the difference between HIV infection and AIDS were still 24% even among house surgeons. Education programmes should be stressed to point out the long duration of asymptomatic HIV infection unknowingly. Importance of taking general precaution to every patients as a potential source, rather

than isolation and avoidance of contact to full-brown cases cannot be over-emphasized in planning teaching programme. Medical students are at the top of the list who must be put under such integrated and comprehensive educational programme and periodic feedback, bearing in mind the fact, that they may spread the misperception which may be hidden uncorrected.

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Evaluation of copper sulfate method for screening anaemia

Myo Khin, Soe Aung, Tin Oo & Thet Lwin

Nuclear Medicine Research Division
Department of Medical Research

The haemoglobin values of 102 blood samples were determined by 3 different observers; the first two observers using the copper sulfate method, and the third observer using the cyanmethaemoglobin method. The sensitivity and specificity of copper sulfate method was calculated at haemoglobin levels of 80 g/l and 100 g/l with comparison to the cyanmethaemoglobin method. At 80 g/l level, it was found to be 100% sensitive with a specificity of 70%. At 110 g/l level, the sensitivity was reduced to 82% while the specificity increased to 100%. The numbers of cases that were either correctly or incorrectly diagnosed by each observer were not significantly different and a good agreement (Kappa statistic = 0.82, 95% Confidence Intervals 0.77 to 0.87) was found between the two observers. The copper sulfate method was found to be reasonably accurate for screening anaemia.

INTRODUCTION

Anaemia is one of the most frequently observed diseases in the world today and a major public health problem. Amongst anaemia, nutritional anaemia is a worldwide problem, and young children and pregnant women are the most affected groups, with an estimated global prevalence of 43% and 51% respectively. Its prevalence in developing countries is the highest and reaches up to 60% in pregnant women and 40% in all groups with the exception of adult males [1]. In Myanmar, haemoglobin surveys carried out by a number of workers have indicated a high prevalence of anaemia among pregnant women and children [2-7]. The findings were later confirmed in a consolidated publication where anaemia of pregnancy was reported to be as high as 71% [8].

It has been suggested that a haemoglobin concentration of less than 130 g/l in adult males, 120 g/l in non-

pregnant women of childbearing age, and 110 g/l in pregnancy is likely to indicate anaemia [9]. Despite its oversimplification, this criterion seems to be a useful guideline for determining the prevalence of anaemia in developing countries.

For any public health programme to be successful in a developing country with limited resources, it is essential that the distribution of available resources be directed to the most vulnerable groups in the population. Even within a vulnerable group, the more anaemic individuals should receive more intensive therapy. If this approach is to be used, a suitable method should be available, whereby health care workers can detect the more anaemic individuals who are to receive more intensive therapy and can subsequently monitor their response to treatment.

However, the internationally recommended method for haemoglobin

determination using photoelectric colorimeter can only be carried out by relatively skilled personnel working under reasonable conditions and is not suitable for use in rural public health programmes. The WHO scientific working group had suggested that a simple screening method for anaemia be developed for use by public health workers in the field [10].

Following the introduction of copper sulfate method for determination of haemoglobin [11], various authors have claimed varying degrees of reliability for screening anaemia in the population [12-14]. This method has been successfully introduced into the health care delivery system in Jamaica where it was found to be moderately accurate [15].

In country like Myanmar, where the prevalence of anaemia is high and resources are limited, we felt that a screening procedure for anaemia should be made available to be used in communities where standard laboratory services are not available. In the present study, an attempt has been made to evaluate the copper sulfate method for its reliability and feasibility as a screening method for anaemia.

MATERIALS AND METHODS

Ten millilitre samples of non-anaemic blood obtained from the Blood Bank, Yangon General Hospital, were diluted with isotonic saline to produce a total of 102 samples within a haemoglobin concentration range of 40 to 130 g/l. Every sample was tested for haemoglobin concentration by three observers. Two observers (laboratory technicians) used the copper sulfate method. Two strengths of copper sulfate solutions were used; the first solution with a specific gravity equivalent to that of blood with a haemoglobin content of 110 g/l, and the second solution with a specific gravity equivalent to a

haemoglobin level of 80 g/l. The true haemoglobin concentration of each sample was determined by the third observer (TO) using the cyanmethemoglobin method [16]. The samples were randomly allocated to the observers and the results were independently recorded. None were aware of the results obtained by the others.

Preparation of copper sulfate solutions

Saturated copper sulfate solution: Two kilograms of fine copper sulfate crystals (Analyser Grade, BDH) were placed in a two-litre bottle. One litre of distilled water was added and the bottle was stoppered and shaken vigorously for five minutes. The solution was immediately decanted off and was filtered through dry filter paper to remove the fine suspended crystals.

Stock copper sulfate solution of specific gravity 1.100: Distilled water was used to dilute 425 ml of saturated copper sulfate solution to a total of one litre. The solution had a specific gravity of 1.100.

Standard copper sulfate solutions: (a) 24 ml of stock copper sulfate solution was diluted to 50 ml with distilled water to obtain a solution with a specific gravity of 1.049 which is equivalent to the specific gravity of blood with a haemoglobin concentration of 110 g/l.

(b) 21 ml of stock copper sulfate solution was diluted to 50 ml with distilled water to obtain a solution with a specific gravity of 1.043 which is equivalent to the specific gravity of blood with a haemoglobin concentration of 80 g/l.

Procedure of the copper sulfate method

Two 100 ml transparent bottles with wide mouths were taken. Each bottle was filled with one of the

two strengths of copper sulfate solution until it was about three-quarters full. A drop of blood was then allowed to fall into the copper sulfate solution with a specific gravity equivalent to that of blood with a haemoglobin content of 110 g/l, from about 1 cm above the surface. The momentum of the drop of blood took it to 1-2 cm below the surface within 5 seconds. The movement of the drop over the next 5-10 seconds (i.e. after the momentum of the fall was lost) was observed. During this short interval the drop of blood behaved in one of the following manner:

- (a) it continued to fall to the bottom without a pause, or
- (b) it remained stationary for a few seconds before finally sinking to the bottom, or
- (c) it rose upward a few millimeters before finally sinking to the bottom.

If it continued to fall, the haemoglobin level was judged to be more than 110 g/l. If it remained stationary before finally sinking to the bottom, the haemoglobin level was regarded to be equivalent to 110 g/l. If it rose upwards a few millimeters before finally sinking to the bottom the haemoglobin level was taken as less than 110 g/l. If the haemoglobin level was less than 110 g/l, the test was repeated in copper sulfate solution with a specific gravity equivalent to a haemoglobin level of 80 g/l. Consequently, the haemoglobin level of every sample was categorized as below 80 g/l, equivalent to 80 g/l, between 80 and 110 g/l, equivalent to 110 g/l, or over 110 g/l.

RESULTS

Altogether 306 observations were made (102 observations by each observer). The observations obtained by the copper sulfate method were compared with those obtained by the

cyanmethemoglobin method. In order to avoid prejudice in classifying the samples, borderline observations (i.e. haemoglobin value of 80 g/l or 110 g/l) were excluded from analysis. Sensitivity, specificity, positive and negative predictive accuracies of the copper sulfate method were then calculated. The correlation between the observations of the first and second technicians was also analysed to see if there was any significant inter-observer variation for the copper sulfate method. Data analysis was performed using CRISP (CRunch Interactive Statistical Package Ver 3.05; Crunch Software Corporation, San Francisco, CA 94116, USA) on an IBM-PC computer.

Observations by the first technician

Out of 102 observations, 7 borderline observations were excluded. The haemoglobin distribution among the remaining 95 cases was as follows: 36 cases (37.9%) had a haemoglobin level below 80 g/l, 48 cases (50.5%) between 80 to 110 g/l, 11 (11.6%) more than 110 g/l. In 22 cases (23.2%) there was a disagreement between the two methods. Of these 22 cases, 14 had haemoglobins levels underestimated by the copper sulfate method, and 8 had haemoglobin levels overestimated. No discrepancies were observed either with the 36 cases with haemoglobin levels of less than 80 g/l or with the 11 cases with a haemoglobin levels of more than 110 g/l (Fig. 1a).

In the 14 cases where the haemoglobin concentration was underestimated by the copper sulfate method, the mean $\pm$ SD haemoglobin concentration as determined by the cyanmethemoglobin method was 86 ± 6 g/l (range 80.3 to 97 g/l). For the 8 cases where the haemoglobin levels were overestimated, the mean $\pm$ SD haemoglobin concentration as determined by the cyanmethemoglobin method was 106 ± 3.3 g/l (range 101 to 109 g/l). The

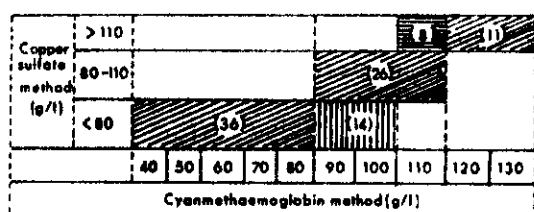


Fig. 1a. Comparison between copper sulfate method and cyanmethaemoglobin method (observer number one)

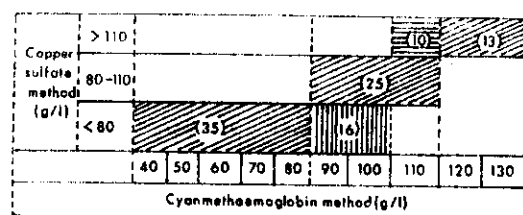
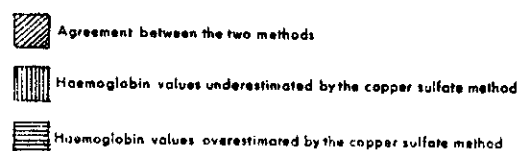


Fig. 1b. Comparison between copper sulfate method and cyanmethaemoglobin method (observer number two)

Figures in parentheses denote number of cases.

maximum error was found to be 20 g/l.

Observations by the second technician

After excluding 3 borderline cases, 99 cases remained for analysis. Of these, 35 (35.4%) had a haemoglobin level below 80 g/l, 51 (51.5%) between 80 and 110 g/l and 13 (13.1%) more than 110 g/l. In 26 cases (26.3%) there was a disagreement between the two methods. Of these, 16 cases had haemoglobin levels underestimated by the copper sulfate method, and 18 had haemoglobin levels overestimated. No discrepancies were observed either with the 35 cases with a haemoglobin level of

less than 80 g/l or with 13 cases with a haemoglobin level of more than 110 g/l (Fig. 1b).

In the 16 cases where the haemoglobin level was underestimated by the copper sulfate method the mean $\pm$ SD haemoglobin concentration as determined by the cyanmethemoglobin method was 85 ± 5.8 g/l (range 80.3 to 99 g/l). For the 10 cases where the haemoglobin level was overestimated, the mean $\pm$ SD haemoglobin concentration as determined by the cyanmethemoglobin method was 105 ± 3.3 g/l (range 101 to 109 g/l). The maximum error was found to be 20 g/l.

Inter-observer variation

All observations of the first and second technician were crosstabulated and agreement statistics calculated (Table 1). A good agreement was

Table 1. Comparison of results of the two different observers using the copper sulfate method for determining the level of haemoglobin

Haemoglobin values as determined by the observer number two

	<80 g/l	80 g/l	>80 g/l- <110 g/l	110 g/l	>110 g/l	Total
<80 g/l	47	1	2	0	0	50
80 g/l	2	0	0	0	0	2
>80 g/l- <110 g/l	2	0	23	1	0	26
110 g/l	0	0	0	1	4	5
>110 g/l	0	0	0	0	19	19
Total	51	1	25	2	23	102

N=102 PEARSON'S R (100 DF)=0.9729 (P=<0.0000)
Kappa statistics (percent agreement = 88.24, percent chance = 35.07, Kappa = 0.82)

found between the two observers (Kappa = 0.82). The number of observations that were correctly diagnosed, overestimated or underestimated were also compared and no significant difference between the two observers was found (Table 2).

Table 2. Number of observations that correctly diagnosed, over estimated, or underestimated the haemoglobin level by each observer

	Observer number one (%)	Observer number two (%)	Total (%)
Haemoglobin level correctly diagnosed	73 (76.9)	73 (73.7)	146 (75.2)
Haemoglobin level over-estimated	8 (8.4)	10 (10.1)	18 (9.3)
Haemoglobin level under-estimated	14 (14.7)	16 (16.2)	30 (15.5)
Total	95 (100)	99 (100)	194 (100)

Chi-square = 0.273 DF = 2 P = <0.872(N.S)

Sensitivity, specificity and predictive accuracies of the copper sulfate method

The sensitivity, specificity, positive and negative predictive values of the copper sulfate method in screening anaemia were calculated for two levels of haemoglobin concentration (Table 3).

DISCUSSION

The copper sulfate method is based upon the observation that the specific gravity of blood is greatly influenced by its erythrocyte volume fraction. Serum protein does not have a large effect on specific gravity, and can thus be neglected [17].

Table 3. Sensitivity, specificity, positive and negative predictive values of copper sulfate method

		Sensitivity (%)	Specificity (%)	Positive Predictive value	Negative Predictive value
Level of haemoglobin (g/l)	80 110	100 83.33	69.8 100	69.1 100	100 57.8

From the present study it was observed that it is essential to let the blood drop from a distance of one cm above the copper sulfate solution. If the drop of blood fell from too great a height, it might either shatter or be carried by its momentum too far towards the bottom of the copper sulfate solution. On the other hand, if the drop fell with too little force, it might fail to break the surface film of the copper sulfate solution. Letting it fall from about 1 cm produced the desirable momentum.

It was also observed that the specific gravity of the drop relative to the solution did not change apparently until the drop had been immersed in the solution for about 15 seconds, but after 15 seconds the specific gravity of the drop of blood began to change and every drop of blood, regardless of its haemoglobin concentration, sank to the bottom. Therefore, it is necessary to record the movement of the drop only between 5 to 10 seconds after delivery.

It was found that both first and second observers could correctly diagnose 100% of cases below 80 g/l and above 110 g/l (Figs. 1a & 1b). Between 80 to 110 g/l there had been some discrepancies between the copper sulfate method and the cyanmethemoglobin method. Within this

range the first technician correctly diagnosed 54% (26/48) of cases, underestimated 39% (14/48) of cases and overestimated 17% (8/48) of cases. Within the same range the second technician correctly diagnosed 49% (25/51) of cases, underestimated 31.4% (16/51) of cases and overestimated 19.6% (10/51) of cases. However, in practical terms, it does not matter too much if subjects with naemoglobin level between 80 to 110 g/l are classified as being less than 80 g/l because they will still be treated as anaemic individuals in either event. Within this context we can safely say that, within 80 g/l to 110 g/l range, 83 per cent (40/48) and 80.4 per cent (41/51) of cases were diagnosed as "being anaemic" by the first and second observers respectively. Of 8 and 10 cases that were overestimated by the first and second observers respectively, none were grossly below 101 g/l.

These results are quite comparable to those of Andrianasolo and co-workers [15] where 82.6 per cent of the patients in their study were correctly diagnosed by the copper sulfate method. The maximum error of the copper sulfate method as observed by both observers in our study was 20 g/l and is equivalent to the finding of Van Slyke and his co-workers [11].

Stone and his colleagues [13] reported that the copper sulfate method was 100% sensitive at both 80 g/l and 100 g/l levels. The specificity was 80% at 80 g/l level and 100% at 100 g/l level. Although our results were slightly lower than those figures, in practical terms they are acceptable.

Stability of the copper sulfate solution

It has been stated that the specific gravity of copper sulfate solution kept in closed glass or plastic containers did not change for over a period of a year and that the solution

kept in open vessels in various environments showed a negligible change in specific gravity over an 8-hour period [13]. Although we have not checked the long-term stability of our solutions, the specific gravity of our solutions did not change throughout our study period which lasted for one month.

Feasibility for field use

Contrary to Topley [14] who stated that the preparation, storage and distribution of copper sulfate solutions required high technical skill, we found it quite simple to prepare and store in the laboratory. When used on a national scale the solutions should be made up at a central point and distributed every few months as suggested by Stone et al. [13]. The test takes no more than two minutes and gives immediate results. Besides, it requires minimum replacement of materials and has proved to be very cheap.

Obviously no single method is perfect for use at the primary health care level. But when one considers in terms of cheapness, sturdiness for field use, ease of operation, independence from either mains electricity or from battery and relative accuracy, the copper sulfate method may prove to be superior to any other method for field use in a developing country.

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**Anti-oedema activity of Nyctanthes arbor-tritis L., Curcuma longa L.
and Plantago major L.**

Aye Than, Mu Mu Sein Myint, Tin Myint & Win Myint

Pharmacology Research Division
Department of Medical Research

Nyctanthes arbor-tritis Linn. (Seik-balu); Curcuma longa Linn. (Nanwin) and Plantago major Linn. (Ahkyaw-baung-tahtaung; Mann-sote-ywet; Htaung-khaung-pwa and Phar-kyaw-ywet) have long been used in folk medicine as anti-oedema agents. Experimental evaluation of anti-oedema property of these plants was studied on carrageenan-induced acute oedema in *in vivo* method using rats. Aqueous extracts of N. arbor-tritis; C. longa and P. major showed a significant anti-oedema activity ($p < 0.005$) at a dose of 3 g/kg. Anti-oedema activities of these plant extracts were observed to be $56 \pm 7.6\%$; $46 \pm 8.4\%$ and $51 \pm 4.9\%$ respectively when compared with that of the control group. Thus, this study has shown that the tested medicinal plants possess anti-oedema activity.

INTRODUCTION

Research on medicinal plants has continued to be an area of major interest for the last several decades. Detailed investigations have been undertaken on several plants for anti-oedema activity. The most common screening model has been the prevention of carrageenan-induced oedema in rats. Curcuma longa L. (Zingiberaceae); Nyctanthes arbor-tritis (Oleaceae) and Plantago major L. (Plantaginaceae) have been reputed to have anti-oedema activity [1-3]. This study has been undertaken on these plants for anti-oedema activity.

MATERIALS AND METHODS

Preparation of the extract

The plants tested and parts used are mentioned in Table 1. Rhizomes of C. longa were purchased from the traditional drug store in Yangon. N. arbor-tritis leaves were collected from Yangon, P. major leaves were collected from Pyin Oo Lwin. All specimens have been deposited at the

herbarium of Pharmacology Research Division, Department of Medical Research, Yangon.

The aqueous extract of each plant was prepared according to the method of Aye Than *et al.* [4]. Crude aqueous extracts were prepared by refluxing 50 g of air-dried leaf or rhizome powder in a round-bottom flask in a boiling water bath using 500 ml distilled water for about 6 hrs. It was filtered using cheese cloth and the filtrate was evaporated to dryness on a boiling water bath. The extract obtained was dissolved in 1% methyl cellulose before administration to respective animals.

Determination of anti-oedema producing activity

Employing the method of Winter *et al.* [5] carrageenan-induced rat paw oedema was compared at 0 and 3 hrs with that of control (1% methyl cellulose). Each drug vehicle (3 g/kg) or acetyl salicylic acid 200 mg/kg were given orally 1 hr before carrageenan challenge. Six groups of 10 rats each were employed. Group

Table 1. Scientific information of plants tested

Scientific name	Myanmar name	Family	Parts used	Distribution	Reputed actions
1. <u>Curcuma longa</u> L.	Nanwin	Zingiberaceae	Rhizome	Cultivated throughout the tropics	Laxative, anthelmintic, anti-oedema, dyspepsia, diuretic
2. <u>Nyctanthes arbor-tritis</u> L.	Seik-balu	Oleaceae	Leaf	Cultivated in many parts of India, Assam, Myanmar, Nepal	Cures fevers, bronchitis, rheumatism. Laxative, remedy for intestinal worms, anti-oedema
3. <u>Plantago major</u> L.	Ahkyaw-baung-tahtaung	Plantaginaceae	Leaf	Temperate and Alpine Himalaya, Assam, Myanmar and westwards to the Atlantic	Cures sores and wounds, diarrhoea, piles, tooth ache, intermittent fevers. Anti-oedema, diuretic

1 received 1 ml/kg of 1% methyl cellulose and served as the control. Group 2 and 3 were treated with 100 and 200 mg/kg acetyl salicylic acid while Group 4, 5 and 6 were given 3 g/kg of the aqueous extracts respectively, 1 hr prior to the sub-planter injection of the carrageenan. The circumferences of the right paws were measured for comparison by means of a marker marked around the oedema, and recorded at 3 hr intervals. Throughout the experiment, the rats were kept comfortably in cages at room temperature (24°C).

Chemical analysis of the aqueous extract

Qualitative identification tests of the chemical constituents present in the tested samples were conducted according to the standard procedures [6-8].

Toxicity testing

Toxicity of the three aqueous extracts was tested by the method of Litchfield and Wilcoxon [9]. An attempt to determine the median lethal dose (LD₅₀) of each extract was made using 3 groups of 10 mice each.

Group 1, 2 and 3 were treated orally with 1, 2 and 3 g/kg respectively. The animals were observed for 7 days.

RESULTS

Neutralisation of oedema-producing effect

In all the rats used in this study, the mean normal paw volume ranged 0.4-0.9 ml. Oedema formation was recorded in the right paws of the control animals treated with the phlogogen, and in those pretreated with the herbal extracts and other known anti-oedema drugs. The right paws of the group pretreated with acute salicylic acid (100 and 200 mg/kg) did not show any sign of oedema formation (Fig. 1). All the three aqueous extracts (3 g/kg) significantly reduced the oedema respectively ($p < 0.005$). Moreover, the aqueous extracts showed percent inhibition of 46 ± 8.4 ; 56 ± 7.6 and 51 ± 4.9 respectively (Fig. 2) whereas the acetyl salicylic acid (200 mg/kg) inhibited 72.8 ± 4.8 percent of oedema.

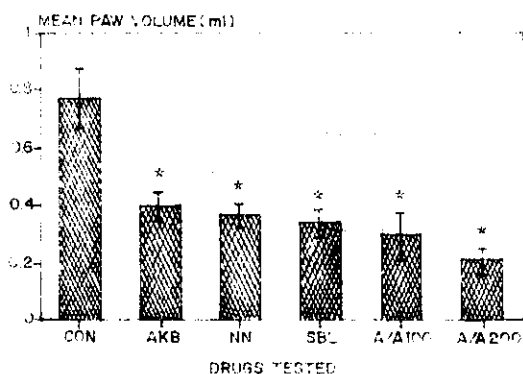


Fig. 1. Effect of aqueous extracts and standard agent on mean paw volume of rats after carrageenan-induced oedema. Each bar represents the mean of observations ($n = 10$) and vertical bars indicate standard errors of the means.

*denotes $p < 0.005$

CON = Control

AKB = Ahkyaw-baung-tahtaung aqueous extract, 3 g/kg

NN = Nanwin aqueous extract 3 g/kg

SBL = Seik-balu aqueous extract 3 g/kg

A/A 100 = Acetyl salicylic acid 100 mg/kg

A/A 200 = Acetyl salicylic acid 200 mg/kg

Chemical evaluation of the aqueous extracts

From the laboratory test carried out, the aqueous extracts contain mainly alkaloids, flavonoid, tannins, saponins and steroids. The results from the qualitative identification tests on the aqueous extract are shown in Table 2. The yield of water soluble extract from the dried rhizome and respective leaf powders were 16 g/100 g from *C. longa*; 23 g/100 g from *N. arbor-tritis* and 13 g/100 g from *P. major* respectively.

Toxicity determination (LD_{50})

It was found not toxic up to 3 g/kg orally in each acute toxicity study. The median lethal dose (LD_{50}) for each aqueous extract on mice was

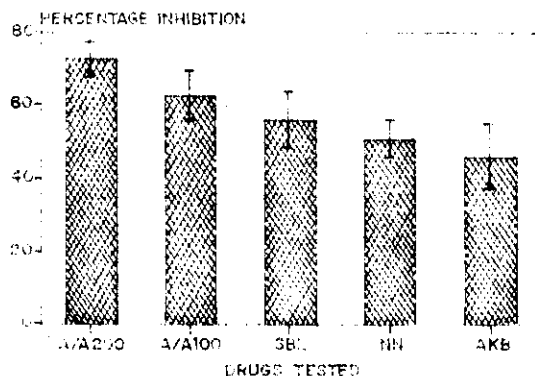


Fig. 2. Anti-oedema effect of aqueous extracts and standard drug on carrageenan-induced rat model. Each bar represents the mean of observations ($n = 10$) and vertical bars indicate standard errors of the means.

AKB = Ahkyaw-baung-tahtaung aqueous extract, 3 g/kg

NN = Nanwin aqueous extract 3 g/kg

SBL = Seik-balu aqueous extract 3 g/kg

A/A 100 = Acetyl salicylic acid 100 mg/kg

A/A 200 = Acetyl salicylic acid 200 mg/kg

greater than 3 g/kg.

DISCUSSION

The present study of the aqueous extracts of the stated three plants on the laboratory animals provides evidence for its usefulness as an anti-oedema substance. The aqueous extracts were shown to produce reduction in carrageenan-induced oedema ($p < 0.005$) in the rat paws. The effect was similar to that mediated by acetyl salicylic acid although the extract took a larger dosage to act.

Results indicate that the aqueous extracts are not acutely toxic even when a massive dose of 3 g/kg are administered. Traditionally *C. longa* rhizome powder, *P. major* and *N. arbor-tritis* leaf paste are also applied topically to the affected fractured

Table 2. Qualitative tests of aqueous extracts of Curcuma longa Linn. (Nanwin), Nyctanthes arbor-tritis Linn. (Seik-balu) and Plantago major Linn. (Ahkyaw-baung-tahtaung)

Sample	Alkaloid	Amino acid	Flavonoid	Glycoside	Saponin	Triterpine steroid	Tannins
<u>C. longa</u> L.	++	+	+++	+++	+	+++	+
<u>N. arbor-tritis</u> L.	-	+	+	++	+	-	+
<u>P. major</u> L.	-	+	+	++	-	-	+

part or oedematous part to alleviate pain and bring about anti-oedema effect. It may be suggested on the basis of these experiments that the herbal extracts could be taken orally without acute toxicity. Chronic effects, however, remain to be investigated.

Qualitative chemical test of the aqueous extracts revealed alkaloids, tannins, steroids, saponins, flavonoids and glycosides. These are presumed to be the active components in the tested plants. Curcumin, the active principle isolated from C. longa, produced significant anti-oedema activity with a low ulcerogenic index [10]. N. arbor-tritis was reported to have anti-oedema activity in a number of acute, subacute and chronic models in rats [11]. Chlorogenic and neochlorogenic acids were isolated from the leaves of P. major and it was postulated that the antisclerotic and anti-oedema effects were probably due to these constituents [12]. Thus, our findings show that the aqueous extracts of the tested plants possess anti-oedema properties. These results support the validity of their use by most of the people in Myanmar. These materials may be used for the treatment of oedematous conditions in human beings.

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Clinical trial to compare the analgesic efficacy of selected Traditional Medicine Formulations (TMF-06, -24, -25) on experimentally induced pain in human subjects

Thaw Zin, *May Aye Than, **Tin Tin Lay, *Aung Naing & ****Mg Mg Wint*

*Pharmacology Research Division

**Clinical Research Unit (Traditional Medicine)
Department of Medical Research

***Department of Traditional Medicine

****Yangon General Hospital
Department of Health

Forty clinically healthy volunteers participated in the study aimed to evaluate the therapeutic efficacy of three Traditional Medicine Formulations (TMF-06, TMF-24 and TMF-25) on experimentally-induced cold compressor stimulation pain. The rationale underlying the study is that these formulations have been produced locally and used extensively as standard analgesics for pain relief at the Traditional Medicine Hospitals and dispensaries as well as through self-medication over-the-counter-drugs by the local community for many years but has yet received little investigative attention regarding efficacy and safety. The study was a placebo controlled double-blind, complete cross-over single dose design using aspirin (acetyl salicylate) as positive standard and was evaluated on three basic pain response parameters namely, pain threshold, pain tolerance and pain sensitivity range. All three formulations showed a significant analgesic efficacy ($p < 0.01$) when compared to placebo (TMF-25>TMF-24>TMF-06). No adverse effects were noted even when given at maximum recommended dose. It was concluded that the three TMFs can be used as an alternative to aspirin for the symptomatic relief of mild to moderate pain.

INTRODUCTION

Pain is one of the commonest affliction encountered in medical practice and serves the useful function of alerting the individual that some component of a physiological system has gone awry. Ideally, pain can be alleviated by the removal of the underlying cause, but in many situations, the stimulus of pain is either not easily defined or not readily susceptible to removal. Therefore, treating pain as a symptom becomes a necessity.

Current techniques for the assessment

of pain and its relief are quite diverse, and each method has its advantages and disadvantages [1,2]. New drugs are usually screened for analgesic activity in animal models before they are tried out in humans. But dependable experiment is lacking and various approaches tried out in animal model have been difficult to reproduce [3].

Clinical trials in humans involve measurement of pain threshold:

- (1) in normal subjects in whom pain has been induced experimentally or
- (2) in patients, usually post-operative

patients, experiencing pain clinically.

Although the analgesic efficacy of narcotic drugs is readily detected in animals, evaluation of non-narcotic agents experimentally is difficult since these compounds can afford relief from pain of slight to moderate intensity. Therefore, careful analysis in humans is required to provide a more meaningful data.

This trial is an attempt to evaluate the therapeutic efficacy of the 3 TMFs, namely TMF-06, TMF-24 and TMF-25 in the treatment of moderate to severe pain in humans.

These 3 TMF: are chosen because:

- (1) They are accepted as potent analgesics by majority of the traditional medicine practitioners.
- (2) They are widely prescribed as analgesic for various types of pain at the Traditional Medicine Hospitals and dispensaries for many years.
- (3) Their low cost and easily availability makes them potential standard analgesics for community use in future.

SUBJECTS AND METHODS

A total of 40 clinically healthy volunteers (24 males and 16 females), aged 20 to 45 years, having no history of peptic ulcer, psychiatric problems, alcoholism or abuse of drugs affecting the CNS (e.g. analgesics, antipsychotics, anxiolytic etc.) or drug medication within the previous 24 hours, were selected after giving a written consent that they were willing to participate in the trial in which they will be receiving repeated painful stimulation and analgesic drugs. The analgesic assay was then validated by doing a preliminary reproducibility study of the pain response parameters (coefficient of variation of $< \pm 15\%$) on the selected volunteers before the

actual study. The main study was a double-blind, complete cross-over single dose design where each subjects were randomly assigned by means of a random table to receive either of the 5 medications, the placebo, aspirin (0.6 g) or one of the 3 TMFs (4 g each) which were administered in powdered form, supplied in a packet labelled and coded by an independent person not participating in the study (i.e. Session I). The pain response was assessed 4 times (i.e. each hand half-hour apart at 1 and 2 hours after drug administration) using the method of Wolff *et al.* [4]. The groups were then cross-over to another drug other than the one previously received and the pain response assessed as before (Session II). This was continued until at the end of 5 sessions, all groups had had received all 5 medications. A wash-out period of at least 48 hours between each study ensured the possibility of carry-over effects. The subjects were also instructed not to eat or drink for a period of 90 minutes prior to taking the medication.

In the method of Wolff *et al.*, induction of pain was done by asking the subject to place his arm up to the wrist in a lukewarm waterbath maintained at 35°C. After exactly 2 minutes, the hand was transferred into the ice-water bath saturated with ice chips and maintained at 0°C and the response parameters quantified in seconds. The subject had been instructed to shout 'one' at the slightest sensation that he feels as hurt (pain threshold), but still keeping his hand in the water until it became really distressing and to shout 'two', but holding on until it became totally unbearable and then to shout 'three' (pain tolerance) and then quickly withdrew the arm and was put back in the lukewarm bath until the pain subside. The time differences between the responses (i.e. 1, 2 & 3) were

noted and the difference between pain threshold (PTH) and pain tolerance (PTR) denotes the pain sensitivity range (PSR).

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

Statistical analysis

All results were recorded on standardised forms and analysed by means of a computer. Paired Student's 't' test was done to find out the significance of difference between the pain response parameters of each active treatment and the placebo, with ($p = 0.05$) taken as the minimal level of significance. Separate analysis of variance (ANOVA) for drug effects were also carried out for each response parameter to test the hypothesis of no difference between treatments.

RESULTS

A total of 40 healthy volunteers (24 males and 16 females) took part in the study and their baseline demographic and clinical characteristics are shown in table 1. All volunteers gave reproducible response of ($< \pm 15\%$) in the 3 basic pain response parameters to be tested.

Table 2 shows the pooled mean scores (in seconds) for all response parameters. No significant difference was seen between the baseline response and the placebo although some "placebo effect" can be seen in the PTR and the PSR. All 4 active treatments were significantly different from the placebo in all pain response parameters ($p < 0.01$) in the order of efficacy as (aspirin > TMF-25 > TMF-24 > TMF-06). Comparative efficacy between treatments showed aspirin

Table 1. Baseline demographic and clinical characteristics of the healthy volunteers participating in the study

1. Total evaluable subjects	-	40
2. Sex - males	-	24
females	-	16
3. Mean age (years)	-	29.2 $\pm$ 8.1
4. Mean height (cm)	-	165.4 $\pm$ 5.8
5. Mean weight (kg)	-	43.9 $\pm$ 4.9
6. Mean baseline responses to pain (seconds)		
- Pain threshold (PTH)	-	20.8 $\pm$ 1.2
- Pain tolerance (PTR)	-	72.1 $\pm$ 5.4
- Pain sensitivity range (PSR)	-	50.5 $\pm$ 5.3

Table 2. Pooled mean scores (in seconds) for the pain response parameters in healthy volunteers

	Onset of pain (pain threshold)	Severe pain	Unbearable pain (pain tolerance)	Pain sensitivity range (PSR)
Baseline	20.8	42.2	72.1	50.5
Placebo	20.4	42.6	92.6	70.9
TMF-06	25.3	52.0	126.9	101.6
TMF-24	29.3	64.4	133.0	103.7
TMF-25	31.0	66.0	140.2	109.5
Aspirin	32.2	72.0	152.5	120.5

to be significantly superior to TMF-06 ($p < 0.001$), but no significant difference was seen between treatments of TMF-24, TMF-25 and aspirin, and between the 3 TMFs studied.

Figure 1 and 2 also show the pooled mean scores (in seconds) for all response parameters. Both figures clearly demonstrate that aspirin and the 3 TMFs studied were consistently more effective than placebo in the order of efficacy (Aspirin > TMF-25 > TMF-24 > TMF-06) and that PTR is a more sensitive index of analgesic efficacy than PTH. Figure 2 also shows that PSR is also a sensitive

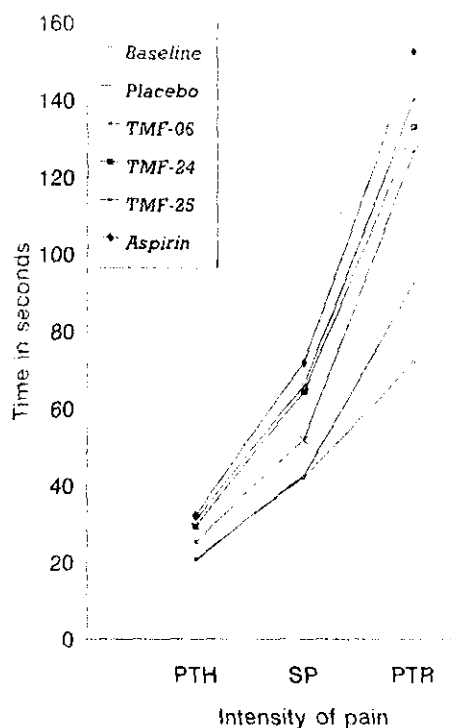


Fig. 1. Comparative analgesic efficacy of the 3-TMFs and aspirin with the normal baseline response and placebo treatment

and reliable index of analgesic efficacy.

No adverse effects were noted by the observers as well as complained by the subjects under study either with aspirin or with the 3 TMFs.

DISCUSSION

In Myanmar, a large segment of the country folk in rural areas were found to rely on traditional drugs and herbal remedies and traditional medicine formulations have been used for symptomatic relief of pain for centuries [5]. Out of the many TMFs, TMF-06 and TMF-25, have been shown not only to be the most prescribed analgesics at the Traditional

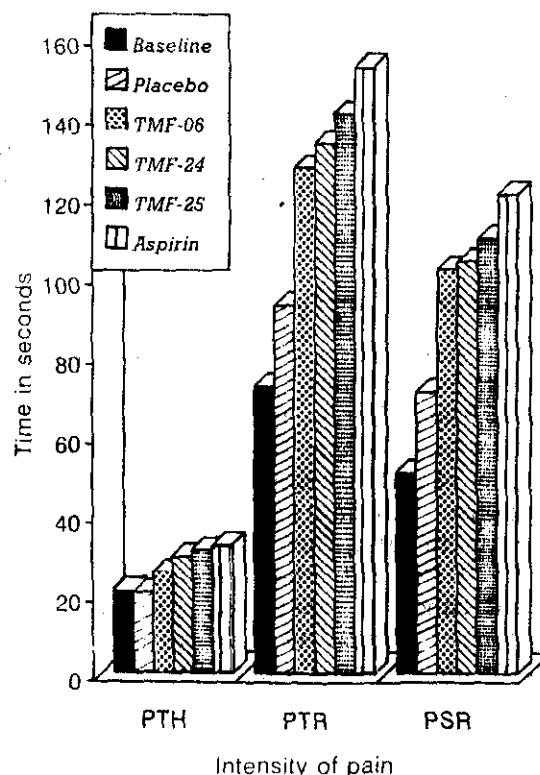


Fig. 2. Pooled means scores (in seconds) for the 3 basic response parameters in healthy volunteers

Medicine Hospitals and dispensaries for moderate to severe pain like painful muscular spasm, joint pain, menstrual pain and traumatic pain, but are also used extensively as over-the-counter analgesics by the local community for mild pain like headache and common cold [6]. However, none of them have undergone scientific evaluation regarding their analgesic efficacy.

The need for a dependable method to test the efficacy of analgesics in man has resulted in many approaches both experimentally and patients [1,2]. The present method was chosen because it has been shown to be the most dependable, economic and least time-consuming pain model

for the analgesic assay in man [3,4] and, in addition, also has the advantage in permitting the direct observation of the 2 pain response parameters (PTH & PTR) and the calculation of the third parameter (PSR). Since it has been shown that many complex variables such as sex, age, ethnic group, psychosocial factors, cultural differences etc. [7-9], have caused large differences between individuals in their responses to pain and analgesic drugs, a complete cross-over design was chosen to keep the inter-individual variation to the minimum. Successive sessions were also spaced sufficiently far apart so as to minimise the carry-over effects (pharmacological interaction) and that the balancing order was preliminary used to counteract psychological interaction, which is presumably less influenced by time [10]. Aspirin was chosen as a positive control because of its established efficacy and safety [11-13], and also because it has been proven to be a comparable standard drug for these 3 TMFs by the previous study [14] where their maximum antipyretic efficacy, which was found to occur between 1-3 hours, justifies the time of study of the present trial under the assumption that their maximum concentration in the blood was reached within that time.

The findings in the present study showing that all three drugs invariably produced more significant changes in pain tolerance than in pain threshold were in agreement with the previous studies which have shown that pain tolerance is a more sensitive index of analgesic efficacy than pain threshold [4,8]. Studies have now shown that the 3 TMFs, like aspirin, possess both analgesic action as well as antipyretic action [14] with TMF-06 having the best antipyretic action while TMF-25 having the best analgesic action. Since these TMFs are easily available locally and cheaply,

and without significant side effects in their recommended dose, this study justifies their use as alternative to non-narcotic analgesics for the symptomatic relief of mild to moderate pain.

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Parasympathetic cardiac activity in Myanmar subjects: Age-related changes

Ohnmar, Nyunt Wai & Khin Myo Chit

Department of Physiology
Institute of Medicine 2, Yangon

Respiratory sinus arrhythmia (RSA) - the variation of heart rate with respiratory activity - is claimed to be the simple and most sensitive measure for the assessment of the cardiac parasympathetic neural function. This study aimed to assess the age-related changes in the cardiac parasympathetic activity in apparently healthy Myanmar subjects. The RSA was measured in 2 different groups (Group 1: 19-25 years, Group 2: 44-60 years), each comprising 30 subjects of mixed sexes. Heart rate variation was assessed by using computerized Electrocardiograph with the subject seated and breathing 50 per cent of his own vital capacity by using Spirometer at a rate of 6 breaths/minute. The magnitude of RSA was significantly lower ($p < 0.01$) in the older age group. It is concluded that the parasympathetic cardiac activity decreases appreciably with age in Myanmar subjects.

INTRODUCTION

The variation of heart rate with the phases of respiration at rest was characterized by increases in the heart rate on inspiration and decreases on expiration [1]. This variation in beat-to-beat interval with a respiratory cycle, commonly referred to as the respiratory sinus arrhythmia (RSA), is enhanced by deep breathing [2]. Since RSA is virtually eliminated by vagotomy [3], blocking the muscarinic receptors [3] or cooling of the vagus nerve [4], it has been suggested that the magnitude of RSA provides an indirect measurement of parasympathetic cardiac activity [4].

It has been shown that the magnitude of RSA decreases with age [2,5] and cardiovascular sympathetic and/or cardiac parasympathetic activity have been reported to change with age [6]. The present study was undertaken to investigate whether the age-related changes in parasympathetic cardiac activity occur in apparently healthy Myanmar subjects, and if so, to what extent.

MATERIALS AND METHODS

Two age groups (19-25 years and 44-60 years) of non-smoking Myanmar adult volunteers of both sexes ($n = 30$ per group) were studied. The history-taking, the clinical examination, 12 lead electrocardiography (Cardiofax-Q, 8450K, Nihon Kohden) and urine sugar testing were done on every subject. Those with cardiopulmonary diseases and diabetes mellitus were excluded from the study. Written informed consent was obtained from all participants. The experiments were performed between 8:00 AM and 2:00 PM, at least 2 hours after the last meal. Subjects were instructed not to consume caffeinated beverages on the day of study. Those who had taken drugs affecting on the cardiovascular and respiratory system the previous day were also excluded.

After resting for 15 minutes in a sitting position, the vital capacity was measured with a closed-circuit spirometer (Griffin, YTK-181-M): the highest of the three readings

was taken as the observed value in litres ATPS. The breathing manoeuvre consisted of breathing 50 per cent of his/her own vital capacity at a rate of 6 breaths/minute, 5 seconds each for inspiration and expiration. After explaining this procedure to the subject, trial runs were carried out until satisfactory tracings were obtained. Thereafter, the subject was allowed to relax and the ECG limb electrodes were attached. The subject with the nose clipped shut, breathed through the spirometer and the respiratory volume and rate were recorded on the chart recorder (CQ 95, WPA, Cambridge, UK). Electrocardiograph recording was taken by using R-R interval mode of the computerized ECG system (Cardiofax-Q, 8450K). Recordings of respiratory signals and ECG were done simultaneously for 1 minute during the breathing manoeuvre. The subject was in the sitting position throughout.

The magnitude of RSA was presented as (a) the E:I ratio: the ratio of mean value for the longest R-R intervals during each expiration to mean value for the shortest R-R intervals during each inspiration and (b) the heart rate difference (beats/minute): the difference between maximal heart rate during inspiration and minimal heart rate during expiration.

Statistical analysis

Data are presented as mean and standard deviations. Student's unpaired 't' test was done for comparison between the groups and the level of significance was set at p value of less than 0.05.

RESULTS

The resting heart rate was found to be significantly lower ($p < 0.05$) in the older subjects compared with the younger ones (72.2 ± 8.9 vs 77.5 ± 9.4

beats/minute respectively), while the mean arterial blood pressure was significantly higher ($p < 0.01$) in the older age group compared with the younger counterparts (92.3 ± 9.6 vs 84.0 ± 8.9 mmHg respectively) (Table 1).

There was no significant difference in the magnitude of RSA between male and female subjects in each group, however, the older age group had a significantly lower values ($p < 0.01$) than their younger counterparts (Table 2, Figures 1 & 2).

DISCUSSION

In the present study, resting heart rate was found to be significantly lower ($p < 0.05$) in the older subjects compared with younger ones. Richardson [7] and many other workers however reported that the resting heart rate did not change with age in adults. It is possible that the age-related difference found in this study may be due to a decline in the sinus node activity in Myanmar. Differences in maturity may also play a role since it was observed that the younger subjects got more excited or emotionally moved during the experiment.

The beat-to-beat variation of heart rate (i.e. the magnitude of RSA) during deep breathing was significantly lower ($p < 0.01$) in the older age group compared with the younger age group. Ageing in man has been reported to be accompanied by a generalised decrease of peripheral nerve function as assessed by nerve conduction velocity studies [8] and histology [9]. Degenerative changes in receptor organ and in brain tissue with concomitant alteration in neurotransmission [10] have also been reported. Thus there may be an age-related impairment in the activity of any one or more of the various portions (receptor, afferent, central

Table 1. The resting heart rate and systemic arterial blood pressures (mean $\pm$ SD) of 2 different age groups

Items	Group - 1 (Age: 19-25 yrs)			Group - 2 (Age: 44-60 yrs)		
	Male	Female	Both sexes	Male	Female	Both sexes
Number	17	13	30	16	14	30
Resting heart rate (beats/minute)	78.2 $\pm$ 9.54	76.6 $\pm$ 9.60	77.5 $\pm$ 9.4	72.0 $\pm$ 7.5	72.5 $\pm$ 10.7	72.2 $\pm$ 8.9*
Resting mean arterial pressure (mmHg)	85.7 $\pm$ 10.4	81.8 $\pm$ 6.2	84.0 $\pm$ 8.9	96.0 $\pm$ 9.8	87.7 $\pm$ 7.3	92.3 $\pm$ 9.6**
Resting systolic blood pressure (mmHg)	114.8 $\pm$ 12.5	106.8 $\pm$ 7.7	111.3 $\pm$ 11.2	122.5 $\pm$ 13.3	112.1 $\pm$ 9.6	117.7 $\pm$ 12.7*
Resting diastolic blood pressure (mmHg)	71.2 $\pm$ 10.6	69.3 $\pm$ 7.3	70.4 $\pm$ 9.2	82.8 $\pm$ 8.4	75.0 $\pm$ 6.8	79.2 $\pm$ 8.5**

* significant at $p < 0.05$

** significant at $p < 0.01$] between 2 groups

Table 2. Sex difference in the magnitude of RSA (mean $\pm$ SD) Group 1 (19-25 yrs) and Group 2 (44-60 yrs)

Group	Sex	Magnitude of respiratory sinus arrhythmia	
		(1)	(2)
1	Male (n=17)	22.9 $\pm$ 6.5	1.31 $\pm$ 0.11
	Female (n=13)	24.4 $\pm$ 7.7	1.36 $\pm$ 0.15
		NS	NS
2	Male (n=16)	14.6 $\pm$ 4.9	1.22 $\pm$ 0.08
	Female (n=14)	13.8 $\pm$ 5.3	1.20 $\pm$ 0.08
		NS	NS

Note:

(1) - Heart rate difference (beats/minute)

(2) - E:I ratio

NS = Not significant

and efferent) of the vagal reflex arc. The effector organ i.e., sinoatrial node itself has been reported to show the age-related decline in

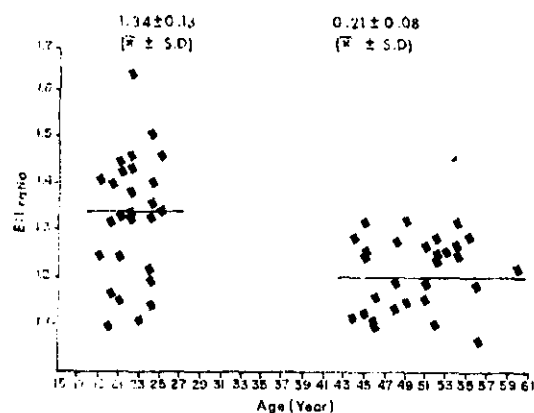


Fig. 1. E:I ratio of young subjects (Group 1, n=30) and old subjects (Group 2, n=30). Bars represent the mean values ($p < 0.01$)

responsiveness to the vagal efferent activity [6]. All these factors may explain the age-related changes in the parasympathetic cardiac activity in Myanmar subjects.

Observed increase in the resting mean arterial blood pressure and a

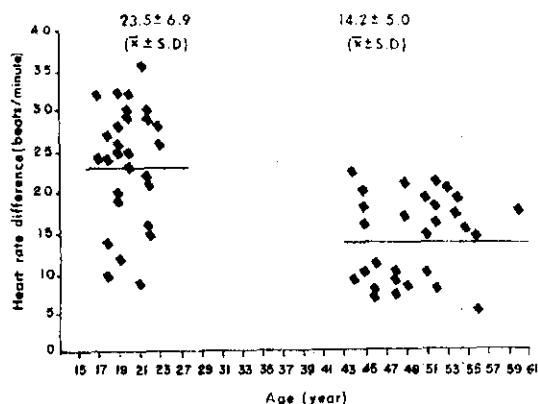


Fig. 2. Heart rate difference of young subjects (Group 1, n=30) and old subjects (Group 2, n=30). Bars represent the mean values ($p < 0.01$)

decrease in the magnitude of RSA are in conformity with the hypothesis that ageing in man is accompanied by dysfunction of baroreflex mechanism leading to a decrease in cardiac parasympathetic activity and a compensatory increase in cardiovascular sympathetic nervous system activity [6].

In conclusion, there is a decrease in magnitude of RSA in Myanmar older subjects. This might suggest the age-related impairment of parasympathetic cardiac activity in Myanmar subjects.

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Russell's viper toxoid: Pattern of antibody response following immunisation with toxoid in rabbits

Aye Aye Myint, Tun Pe, Nu Nu Aung & Kyi May Htwe

Immunology Research Division
Department of Medical Research

A prospective study of the development and persistence of antibody following immunisation with Russell's viper toxoid was carried out in rabbits. Two immunisation schedules, each consisting of 3 injections of the toxoid given at 0, 4 and 8 weeks and at 0, 6 and 30 weeks were studied. Booster injections were given at 24 and 60 weeks in the former and at 50 weeks in the latter. The pattern of antibody response of the two immunisation schedules was comparable. Peak antibody response was maintained for 8 weeks after the third injection. No antibody response was observed following boosting at 30, 50 and 60 weeks. Use of aged toxoid and timing of boosting may play an important role in induction of subsequent antibody responses.

INTRODUCTION

Russell's viper (RV) bite is a common occupational hazard of farmers which carries a mortality rate of 10%. Most RV bite patients had signs of systemic envenoming at the time of arrival at the hospital. In spite of treatment with potent antivenom, mortality rate remains high. Active immunisation with toxoid to the risk population may help to lessen the severity of envenoming. With that view in mind, a formalin-treated RV toxoid had been developed and its immunogenicity was tested in monkeys [1] and in human volunteers [2]. However, the pattern of antibody response was followed up to 24 weeks only. In the present communication, the development and persistence of antibody following active immunisation was studied in rabbits up to 64 weeks.

MATERIALS AND METHODS

Russell's viper toxoid was prepared by stepwise formalinisation method [3]. The protein concentration of

the final product was adjusted to 2 mg/ml, aliquoted and stored at +4°C. Two immunisation schedules were carried out; schedule (1) 4 weekly injection of 3 doses of the toxoid followed by 2 boosters at 24 and 60 wks, and in schedule (2) injections were given at 0, 6, 30 and 50 wks. The ages of the first dose of the toxoid of schedule (1) and (2) were one and three months respectively. Subsequent immunisations were carried out with the aged toxoids. Two rabbits per schedule were used. Serum samples were collected at 4 wk intervals up to 24 wks and at 6 wk intervals up to 64 wks following immunisation. Venom antibody levels were measured by enzyme immunoassay technique [4]. Peak serum samples collected at 6 and 16 wks after the third injection of the toxoid (schedule 1) were tested for neutralising activities to venom.

ED₅₀ neutralising dose of antivenom was estimated as the amount of antivenom in microlitres which will neutralise 50% of the activity. Various amounts of antivenom were mixed

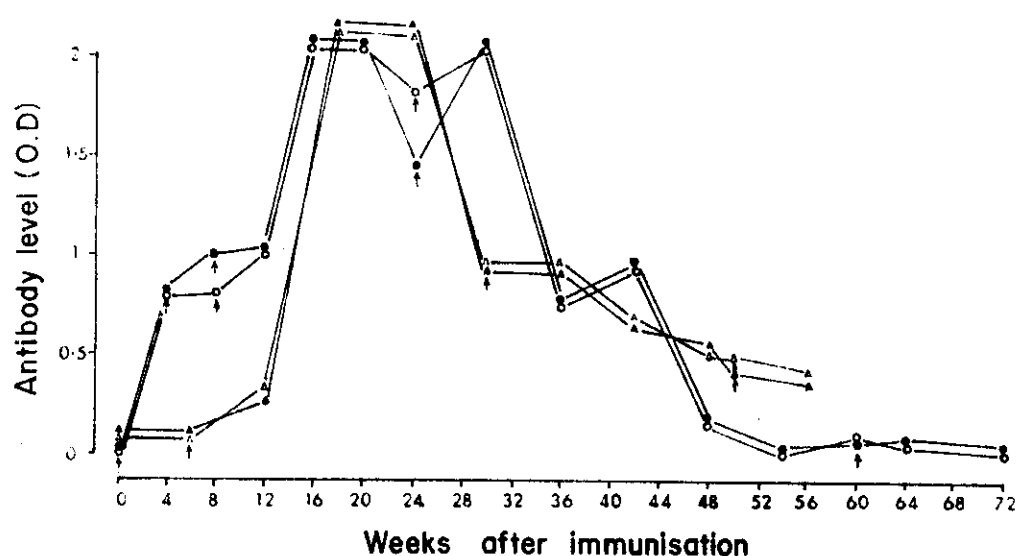


Fig. 1. Antibody response of rabbits immunised with two different schedules of RVV toxoid. Open and closed circles represent two rabbits immunised (↑) with toxoid at 0, 4, 8, 24 and 60 weeks. Open and closed triangles represent two rabbits immunised (↑) with toxoid at 0, 6, 30 and 50 weeks.

Table 1. ED₅₀ effective neutralising doses of the two peak rabbit antivenoms

Preparation	1 MLD ul	1 MHD ul	1 MND ul	1 MDD ul	1 MCD ul	1 MCPID ul
Rabbit antivenom (6 weeks)	40	80	80	20	5	1.25
Rabbit antivenom (16 weeks)	75	80	80	20	5	1.25
Monospecific antivenom 86608 B*	1.56	1.56	3.125	3.125	0.019	1.56

MHD = Minimum haemorrhagic dose

MCD = Minimum coagulant dose

MCPID = Minimum capillary permeability increasing dose

MND = Minimum necrotic dose

MDD = Minimum defibrinogenating dose

MLD = Median lethal dose

*Included for reference

with a fixed amount of venom, incubated at 37°C for 30 min and injected into mice or rats accordingly.

RESULTS

The antibody responses of two immunisation schedules are shown in figure 1. The pattern of antibody response following 3 injections of the toxoid in schedule (1) and two injections in schedule (2) was comparable except early peaking of antibody in the

former. Peak antibody level was maintained for 8 wks only. Further boosting at 30, 50 and 60 wks failed to induce antibody response. Results and neutralisation of different biological properties of the venom by the rabbit antivenoms are shown in table 1. Weak neutralisation of lethal, necrotic, coagulant and haemorrhagic activities of venom was noted.

DISCUSSION

The development and persistence of

antibody following immunisation with the RV toxoid was studied for 64 weeks. A similar pattern of antibody response was observed following 3 injections of the toxoid in monkeys [1] and in human volunteers [2]. Had the latter studies been extended to 64 weeks, waning of antibody with time will be observed as in this case. Antibody peaked at 6 weeks after the third injection; maintained at it's peak level for 8 weeks and began to decline in 24 wks. A similar antibody peak could be achieved with two injections of 6 weeks apart (schedule 2). A booster injection given at the decline of antibody induced antibody response whereas subsequent injections at low antibody levels failed to do it suggested that timing of boosting may play a role in induction of subsequent antibody response. On the other hand, use of aged toxoid may account for poor antibody response. It has been observed that an aged toxoid of 50 weeks old was a poor immunogen (unpublished observation).

It is essential to have a high resting antibody level in Russell's viper toxoid immunised subjects. When snakebite occurs, immediate neutralisation of venom with preformed antibody is required. Even neutralisation of a fraction of venom by antibody will be helpful in reducing the degree of envenoming before antivenom could be given. In order to achieve this goal, frequent boosting will be required (or) toxoid which will induce long lasting antibody response with minimum injection will be ideal.

The possibility of active immunisation of man using venom/liposomes activated both parenterally and orally to experimental animals is in progress [5,6].

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SHORT REPORT

Neurotoxicity of artemisinin compounds

Tin Shwe, **Khin Hlaing, *Ko Ko Hla*

*Clinical Research Unit (Cerebral and Complicated Malaria)
Department of Medical Research

**Township Medical Officer (Retired), Lashio

***Institute of Medicine 2

The safety of artemisinin group of drugs has been in question because the early preclinical and clinical studies were insufficient to satisfy international drug regulatory standards and requirements. This has necessitated repetition and augmentation of studies to validate the earlier findings.

Recent studies in rats and dogs have demonstrated neurotoxicity following administration of artemether or arteether at dosage regimens higher than those employed in humans. Following regimens of 10 mg/kg/day x 8 day transient neurological defects occurred in dogs while the dosage of 20 mg/kg/day produced a progressive neurological syndrome and death in most of the animals [1,2].

During 1994-95 one of us had the opportunity to visit North Okkalapa, Mawlamyine, Dawei, Beik, Mudon, Mandalay, PyinOoLwin, Myitkyina, Moekaung, Thayawady and Lashio Townships to study the drug utilization pattern of artemisinin and its derivatives in Myanmar. During these visits he had met over 70% of the doctors of the townships and held discussion on management of severe and complicated malaria. During these discussions the possible neurotoxicity following the use of artemether was discussed.

In the 10 years experience of our unit (Cerebral and Complicated Malaria), Department of Medical Research with artemisinin compounds and the experience of the doctors from the

above townships, the following five cases may be neurotoxicity due to artemisinin compounds.

Case 1

A 23-year-old female was admitted for malaria and was treated with quinine standard dose. Her parasite load did not fall at 72 hours. Therefore, she was put on oral halofantrine 500 mg 6 hourly for 3 doses. She became comatose at another 24 hours and was put on injection artemether 600 mg total dose and mefloquine 750 mg via nasogastric tube. Parasites were cleared at 36 hours. But she remained unconscious for another 12 days. When she recovered, she was in vegetative state and transferred to Neuro Medical Ward of Yangon General Hospital. All investigations including CAT Scan were done and no abnormality was detected. Tests for viral antibodies were also negative. She showed no response to treatment and died 1 month later.

Case 2

A 39-year-old male admitted to North Okkalapa General Hospital for diarrhoea and fever of three days duration. He became unconscious while in hospital and his M.P report showed Pf >100,000/cu mm, and was treated with artesunate and mefloquine standard dose. His fever clearance time was 48 hr, parasite clearance time was 60 hr and time to regain consciousness was 72 hr. He deve-

loped stricture urethra and cystostomy was done. He also developed herpes zoster at genitalia. He was later proved to be HIV positive.

Although he regained consciousness at 72 hrs his mental recovery was slow. For this reason he was treated with domaxethazone 4 mg tabs 2 days - to get full recovery.

Case 3 and 4

Two Chinese males aged 45 and 50 respectively came to Lashio clinic with marked ataxic gait. According to the history they developed these symptoms after receiving the six injections of artemether in Muse, a border town close to China.

Case 5

Another Chinese male aged 18 came to Lashio clinic on 13-8-95 with weakness of both lower limbs after receiving the artesunate tabs for his fever.

All these three patients took a long time for recovery.

In this report five patients who had developed some neurological manifestations following the use of artemisinin compounds were recorded. As the clinical signs and symptoms reported in this study are not common to each other it is difficult to differentiate whether these are due to

a part of the disease process or due to the effect of the drugs. In humans over two million malaria cases are estimated to have been treated with artemisinin or its derivatives. In these patients neurotoxicity had not been reported [3]. Hence the above signs and symptoms were more likely to be the result of the disease malaria, rather than due to the effect of artemisinin compounds.

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SHORT REPORT

Specificity of antibodies to hepatitis B surface antigen

Myo Khin & Khin Aye Tha

Nuclear Medicine Research Division
Department of Medical Research

The hepatitis B virus (HBV) has infected more than 2,000 million people today and is one of the major diseases of mankind [1]. Following infection with HBV, hepatitis B surface antigen (HBsAg) appears in the serum. In those who recover completely from the infection, antibodies to HBsAg (anti-HBs) appear in the serum corresponding with the disappearance of HBsAg.

It is known that HBsAg contains several antigenic determinants: termed "a", "d", "y", "w" and "r". While some 10 subtype combinations are recognized, "a" is the common antigenic determinant in all the subtypes and the most common are "adw", "adr" and "ayw". The "y" and "r" determinants are, respectively absent from Far-East Asia and from Africa [2].

In recent years the availability of HBV vaccine in Myanmar has allowed widespread use of the vaccine among the general population. Also, HBV infection rate among the general population is high as reflected by the high percentage (40%) of anti-HBs among the blood donors. The question arises whether the antibodies to HBsAg resulting from infection or immunization is specific to the local strain(s) of HBV.

We undertook a study to determine the specificity to the antibodies of HBsAg in 13 subjects with detectable levels of antibodies to HBsAg in their sera. Seven subjects had been immunized with three doses of recombinant HBV vaccine (Engerix-B, SmithKline Beecham Biological, Rixen-

sart, Belgium) and six did not receive vaccination and were regarded to have acquired antibody naturally following infection with HBV.

The purified HBsAg prepared locally from the sera of HBsAg positive blood donors was used as part of the HBV vaccine production programme as the inhibitor. For control, phosphate-buffered saline (PBS), pH 7.2 was used. The inhibition procedure was as follows. 190 μ l of test serum was incubated with 10 μ l (0.5 μ g) of purified HBsAg for one hour at 37°C. The same volume of test serum was also incubated with 10 μ l of phosphate-buffered saline (PBS), pH 7.2 under the same condition. To determine the anti-Hbs levels the solid-phase radioimmunoassay (SPRIA) kits from China Institute of Atomic Energy, Beijing, China, supplied by the International Atomic Energy Agency were used. The assay procedure was as follows. 200 μ l of samples and positive and negative controls were incubated with HBsAg coated beads for one hour at 45°C in the water bath. The beads were washed with distilled water and 200 μ l of HBsAg ^{125} I was added. After incubating at 45°C for next one hour, the beads were washed again with distilled water and their radioactivity was counted using the multi-well gamma counter (RIASTAR A 5410, Packard Company, USA). The anti-Hbs levels were expressed as Sample Ratio Units (SRU) (sample signal counts per minute (cpm) divided by the test kit negative mean cpm).

We found that over 80% of the anti-

HBs activity was reduced following incubation with the purified HBsAg. The overall mean ($\pm$ S.E.) reduction was 95% ($\pm$ 13%). This was highly significant ($P < 0.001$, Wilcoxon signed rank test) as compared to controls where less than 16% reduction (mean $\pm$ S.E., 10% $\pm$ 2.4%) was seen. The mean anti-Hbs levels before and after inhibition with HBsAg or PBS were not significantly different between the immunized and natural immunity groups (Table 1).

Table 1. Antibody to hepatitis B surface antigen levels expressed as Sample Units (SRU) in serum samples

Subjects	Antibody to HBsAg levels SRU (mean $\pm$ S.E.)		
	Neat serum	Inhibition with HbsAg	Inhibition with PBS
Those immunized with three doses of HBV vaccine (n=7)	67.19 ^a ± 14.25	2.27 ^b ± 0.76	65.33 ^c ± 13.4
Those who acquired natural immunity (n=6)	69.40 ^a ± 17.85	3.19 ^b ± 3.26	60.56 ^c ± 17.36

a b c $p > 0.05$ (Wilcoxon signed rank test)

In the present study, we found significant inhibition of anti-HBs activity by purified HBsAg in serum samples of immunized subjects and also in those who had acquired natural immunity. The percentage of reduction of over 80% is also the same in both groups. Werner and co-workers studied the specificity of antibodies to HBsAg in serum following a full course of vaccination with Heptavax-B. They concluded that antibodies to HBsAg in serum were considered to be specifically inhibited by purified

HBsAg if the anti-HBs activity was reduced by 50% or more [3].

The antigenic determinants of HBsAg of Myanmar population is yet to be determined. However, the antibodies to hepatitis B surface antigen of our subjects appear to be biologically specific. Those who acquired natural immunity are likely to be infected with the local strains of HBV. Those with acquired immunity had been immunized with the commercial HBV vaccine. The specificity of the developed antibodies to purified HBsAg is probably by virtue of the antibody to the group reactive "a" determinant. This has been proven when the HBV was of the same subtype and has been inferred when the HBV is of a different subtype [4]. From the present study, we conclude that the antibodies to HBsAg produced following natural infection or active immunization was specifically inhibited by surface antigen of the local strain of HBV.

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