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A study of lipid profile in Myanmar smokers and nonsmokers

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This study was carried out to see the levels of serum lipids in smokers and nonsmokers. A total of 155 healthy subjects from the staff of the Institute of Medicine II, Yangon were studied. Among the 155 subjects, 96 were nonsmokers and 59 were active current smokers, smoking 1-9 cigarettes a day. Over night fasting samples of serum total cholesterol, serum HDL cholesterol, serum triglycerides and serum phospholipids were measured. It was found that smokers had significantly higher level of serum total cholesterol than nonsmokers.

INTRODUCTION

Cardiovascular diseases can be regarded as a major health problem throughout the world. The association of abnormalities of serum lipids with cardiovascular diseases such as atherosclerosis and ischaemic heart disease is well documented [1-4]. Smoking is said to be one of the major risk factors that can cause ischaemic heart disease. This study was designed to see the blood lipid levels of smokers and nonsmokers, so as to know the probable dangers of smoking habit in Myanmar. The information from this study will have important implications on the great debate for necessity of public health campaign designed to modify smoking behaviour in Myanmar.

MATERIALS AND METHODS

One hundred and fifty five apparently healthy subjects (96 nonsmokers and 59 active current smokers, smoking 1-9 cigarettes a day) of different age, sex and socio-economic classes

from the staff of the Institute of Medicine II, Yangon were selected for the study.

The subjects (age between 15-60 years) free from cardiovascular diseases (excluded by history and clinical examination) and do not take any treatment or medication that might affect their lipid level were studied. They were on their usual diet which was not too rich in fat or cholesterol.

Each participant was interviewed and the questionnaire form was filled by asking the subject about his/her occupation, past and present illness and a dietary history. The subjects were asked whether they were current smokers or nonsmokers. Fasting blood samples were collected and serum total cholesterol (Zlatkis, Zak and Boyl 1953), serum HDL cholesterol (Lopes Virella *et al.*, 1977), serum triglyceride (Foster and Dunn 1973 and Flechter, 1968) and serum phospholipids (Connerty *et al.*, 1961) were measured within one hour after collection of samples. Standard lipid

solution was included in each batch of determination of samples and duplicate of each sample were measured.

RESULTS

The mean and SD values of serum lipids of 155 subjects were shown in Table 1 and the mean and SD values of smokers and nonsmokers were shown in Table 2. Student's 't' test for observations was done to determine

Table 1. Mean and SD in mmol/L of serum lipids in 155 subjects

Serum lipids	Mean	SD
Total cholesterol	4.654	1.86
HDL cholesterol	1.372	0.69
Triglycerides	1.118	0.59
Phospholipids*	9.575	2.65

*in mg/dl expressed as P

Table 2. Mean and SD of serum lipids in mmol/L of 59 smokers and 96 nonsmokers

Serum lipids	Smokers	Nonsmokers
Total cholesterol	4.57±1.97	3.61±1.43
HDL cholesterol	1.32±0.82	1.45±0.72
Triglycerides	1.24±0.71	1.09±0.65
Phospholipids*	10.74±10.1	11.32±11.0

*in mg/dl expressed as P

any significant difference between means. Only serum total cholesterol values showed significant difference ($P<0.05$) and other values showed no significant difference between smokers and nonsmokers.

DISCUSSION

Thanet Israsena *et al.* (1974) reported in their study of lipid profile of

normal Thai subjects, 26 smokers, some of them smoking as much as 10 to 20 cigarettes per day for 20 years had abnormal levels of cholesterol, triglycerides and phospholipids. Irso Stubbe *et al.* (1982) indicated that there was no significant changes in plasma triglycerides, cholesterol and LDL concentration in 10 habitual smokers who were monitored for 6 weeks after they had stopped smoking but there was a significant correlation between the rise in high density cholesterol concentration. Helen Pozner *et al.* (1970) stated that fasting plasma turbidity, total cholesterol and β -lipoproteins were significantly increased in heavy smokers. The fact that our study did not demonstrate any difference in all serum lipids except total cholesterol between smokers and nonsmokers may be due to lower level of daily cigarette consumption (1-9 cigarette per day) and usual background diet which is not too rich in saturated fats.

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**Comparing the efficacy of mefloquine-artesunate combination with
that of mefloquine and artesunate as single agents in
uncomplicated falciparum malaria**

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To find out whether the combination regimen of mefloquine and artesunate tablets improves the cure rate in uncomplicated falciparum malaria compared to the use of mefloquine and artesunate as single agents, a prospective, randomised, unblind controlled study was done between May and November 1991, on one hundred and eighty cases of acute uncomplicated falciparum malaria. The patients were randomised to the following three drug regimens:- (1) Mefloquine - 1000 mg stat, (2) Tab Artesunate 600 mg over 48 hours, (3) Mefloquine 1000 mg plus tab Artesunate 600 mg over 48 hours. The regimen of mefloquine as a single agent gave a cure rate of 83.3% (with $R_1=15\%$ and $R_2=1.7\%$), $PCI=52.15\pm55.2$ hours and $FCI=23.98\pm59.87$ hours. Tab artesunate gave a cure rate of 75% (with $R_1=25\%$), $PCI=33.16\pm5.15$ and $FCI=23.4\pm22.7$ hours. The combination regimen gave a cure rate of 98.27% with only one patient recrudescing at R_1 level, $PCI=35.11\pm29.16$ and $FCI=8.15\pm10.34$ hours. The side effects were not much increased by using the combination. No cardiovascular effects were noted. The combination is superior to that of the single agents.

INTRODUCTION

The emergence of strains of Plasmodium falciparum resistant to chloroquine and other widely used anti-malarials have created serious problems in the control of malaria. The spread of multidrug resistant falciparum malaria make it necessary to develop alternative treatment regimens.

When mefloquine was first introduced it was effective against multidrug resistant strains of Plasmodium falciparum. Clinical trials of mefloquine in the early 1980's in different areas of the world showed a high efficacy (89-100%); (WHO, 1984). In Myanmar in 1984 mefloquine response

rate was 100% [1]. It has fallen to 82% in 1990 with a regional difference in response rate between the patients contracting falciparum malaria from the Kayin State (along the Thai-Myanmar border) with a cure rate of only 63.63% compared to those from other parts of Myanmar with a combined cure rate of 80.62% [2].

In Thailand in 1985-1986, MSP (Mefloquine-Sulphadoxine-Pyrimethamine combination) cured 93% of 5192 patients with falciparum malaria on the Thai-Myanmar border [3]. Four years later it was reported to be 70.8% of 375 patients at the same location [4]. Artemisinin derivatives have been shown to have rapid parasite

clearance rates but with high recrudescences between 10-50% [5-8]. Data from Thailand and our own studies confirmed the above findings [9]. For a new drug, these results are therefore not satisfactory for curative purposes.

Drug combination studies in *Plasmodium falciparum* infected rodent models [10] as well as in vitro studies on chloroquine resistant as well as sensitive *Plasmodium falciparum* strains have shown that there is a potentiative synergism between artemisinin derivatives and mefloquine [11]. Our own experience with this combination in Human Cerebral Malaria has been impressive, with no recrudescences in all 58 patients who survived [12]. In another of our in vivo studies four cases of uncomplicated falciparum malaria, were unresponsive to old drugs like chloroquine, sulphadoxine-pyrimethamine and quinine; and recrudescing at R₁ level to mefloquine as well. These cases still remained unresponsive to tablet artesunate and again to halofantrine and even to the intravenous quinine-oral tetracycline combination, but were eventually cured with mefloquine-artesunate combination [13]. Hence this study is done to confirm if the combination significantly improves the cure rate in uncomplicated falciparum malaria compared to the use of mefloquine and artesunate as single agents.

PATIENTS AND METHODS

Between May and November 1991, a prospective, randomised, unblind controlled study was done on 180 male patients between the ages of 18 to 42 years, admitted to the Defence Services General Hospital, Mingaladon with uncomplicated falciparum malaria. They were

included into the study if they had asexual forms of *Plasmodium falciparum* in their peripheral blood with an initial parasite count of at least 500 per microlitre and not exceeding 200,000 per microlitre; and were excluded if they have taken any form of antimalarial drugs within the past 14 days and also if they had any concomitant illnesses or any form of severe malaria as defined by W.H.O. criteria (1990) [14]. Patients whose initial or subsequent blood smears showed mixed infection (falciparum and vivax) were also excluded from the study. They were kept in hospital for 28 days. The hospital is situated in an area where no active transmission of malaria exists.

A thorough history was taken and physical examination was performed on each patient at the time of admission and regularly throughout the observation period. The patient's body weight was measured at the time of admission and weekly. Temperature was taken six hourly till normal and then daily. Parasite count was done six hourly till negative and then daily up to day 7 on days 14, 21 and 28, and whenever the patient was symptomatic with fever.

Routine baseline investigations including blood for Hb%, total and differential wbc count, platelet count, erythrocyte sedimentation rate, reticulocyte count, urea, sugar and bilirubin were done. Urine and stool for routine examination were also done. These tests were repeated on days 7, 14 and 28. E.C.G. was taken before giving the drug and daily up to day 5.

The patients were randomised to the following three drug regimens:- (1) Oral mefloquine 1000 mg stat; (2) Oral sodium artesunate tablet 100

mg 12 hourly for 2 doses followed by 50 mg 12 hourly for 8 doses-total 600 mg; (3) Oral mefloquine and sodium artesunate as above given simultaneously. Drug administration was closely supervised and observed for any vomiting within 2 hours of administration.

Drug treatment was considered curative if there was a clearance of parasitaemia with no recrudescence during the 28 days follow up. The W.H.O. terminology was used in this paper to classify recurrences of malaria as R₁, R₂ and R₃. Additional parameters of drug efficacy were fever clearance time (FCT), measured from the time of drug administration until the oral temperature was 37°C or less and remained so for at least 48 hours while the parasite clearance time (PCT) was from the time of drug administration until an examination of 200 fields on a thick smear revealed no parasites. Quantification of parasites was expressed per microlitre, calculated by counting the number of parasites against 200 wbc's and multiplying by 30 (Average wbc count was taken as 6 x 10⁹ per litre).

Side effects were enquired by means of a check list covering all systems. All data were recorded on case recording forms, which were designed to be analysed by the computer using SPSS and EPI-INFO softwares.

RESULTS

Although all 180 patients were evaluable for the study, two patients from regimen 3 were excluded due to subsequent appearance on days 16 and 22 respectively of Plasmodium vivax.

Comparison of the three groups for the baseline characteristics such as age, body weight, initial temperature

revealed no significant differences. Initial parasitaemia was significantly higher, in spite of strict randomisation, in the group that received the mefloquine-artesunate combination. Hepatomegaly and splenomegaly was detected in all three groups, mild to moderate enlargement of the spleen ranging from 1-4 cms and mild enlargement of the liver of 102 cms were observed. Baseline haematological and biochemical parameters were also comparable (Table 1).

Table 1. Clinical and laboratory baseline characteristics

Regimens	M (n=60)	A (n=60)	M+A (n=58)
1 Age (years)	22.05	21.73	21.25
Mean (S.D.)	(4.83)	(0.59)	(3.412)
2 Body weight(Kg)	48.3	46.53	46.05
Mean (S.D.)	(5.39)	(3.48)	(4.96)
3 Initial temperature °C	38.25	37.56	37.66
Mean (S.D.)	(2.74)	(3.8)	(4.6)
4 No. of patient with			
splenomegaly	48	37	39
hepatomegaly	48	40	38
5 Parasite count per microlitre			
Geometric mean	4299.5	7105.8	14376.6
Range	240- 48,000	220- 96,000	180- 160,000
6 Laboratory data:			
Hb% Mean (S.D.)	9.10 (2.28)	5.82 (2.6)	9.75 (1.71)
Total wbc count x 10 ⁹ per litre	5.76 (1.9)	170.0 (38.04)	5.56 (1.92)
Platelet count x 10 ⁹ per litre	183.20 (72.11)	170.0 (38.04)	192.6 (88.93)
ESR mm/hr	55.9 (34.31)	59.93 (41.93)	50.9 (45.5)
Blood urea m mol/litre	4.36 (2)	4.59 (1.05)	4.46 (2.08)
Blood sugar m mol/litre	6.475 (1.71)	7.42 (3.01)	6.32 (2.09)

Table (1). Contd.

M = Mefloquine 1000 mg stat; A = Artesunate 100 mg 12 hourly for 2 doses followed by 50 mg 12 hourly for 8 doses; M+A = Combination of the two

Table 2. Response of treatment compared

Regimens	M (n=60)	A (n=60)	M+A (n=58)
1 Parasite clearance time(hours) Mean (S.D.)	52.15 (55.2)	33.16 (5.15)	35.11 (29.16)
2 Fever clearance time(hours) Mean (S.D.)	34.98 (59.8)	23.4 (2.7)	8.15 (10.34)
3 No. of patients cured at 28 days(%)	50 (83.3)	45 (75)	57 (98.27)
4 Recrudescences			
R ₁ No. (%)	9 (15)	15 (25)	1 (1.8)
R ₂ No. (%)	1 (1.7)	0	0

M = Tab. Mefloquine 1000 mg stat; A = Tab. Sodium Artesunate 100 mg 12 hourly for 2 doses followed by 50 mg 12 hourly for 8 doses; M+A = Combination of the two regimens (R₁, R₂ = recrudescence according to WHO classification)

Table 3. Comparing the side effects

Side effects	M (n=60)	A (n=60)	M+A (n=58)
No. (5) of patients with:			
Dizziness	22 (36.66)	10 (16.66)	16 (26.6)
Nausea	8 (13.33)	2 (3.3)	9 (15)
Vomiting	2 (3.33)	1 (1.6)	6 (10)
Abdominal pain	11 (18.33)	3 (5)	24 (40)
Diarrhoea	3(5)	1(1.6)	8(13.3)
Pruritis	0	0	1(1.6)
Myalgia	2 (3.33)	0	6 (10)
Arthralgia	0	0	1(1.6)
Insomnia	9(15)	2(3.3)	6(10)
Headache	1 (1.66)	0	0

Table 4. Results of laboratory investigations on 20 patients given mefloquine-artesunate combination

Day	0	7	14	21	28
Mean (S.D.)					
1. Hb %	8.9 (2.76)	9.93 (1.62)	11.37 (2.39)	11.77 (1.23)	12.79 (1.94)
2. wbc count x 10 ⁹ /L	4.72 (1.29)	6.01 (1.64)	6.48 (1.60)	6.2 (1.97)	7.05 (3.88)
3. ESR mm/hr	55.43 (32.74)	39.5 (19.86)	28.92 (20.49)	32.1 (21.1)	10.83 (7.86)
4. Reticulocyte count %	0.8 (0.9)	1.23 (0.9)	0.4 (0.39)	1.1 (0.8)	0.88 (1.3)
5. Platelet count x 10 ⁹ /L	136 (36.3)	141 (63.5)	240 (34.7)	206 (13.6)	184 (58.7)
6. Total bilirubin (micro mol/L)	21.26 (22.62)	13.14 (3.07)	14.16 (2.2)	16.08 (3.62)	13.2 (1.04)
Direct bilirubin (micro mol/L)	13.14 (19.4)	7.52 (2.69)	7.29 (2.3)	8.43 (3.06)	4.93 (2.59)
7. Alkaline phosphatase (K.A. Units)	6.21 (2.4)	5.8 (0.82)	5.7 (0.81)	6.09 (1.05)	5.27 (0.63)
8. Blood usugar (m mol/L)	4.67 (1.0)	4.29 (0.16)	4.5 (1.08)	4.08 (0.61)	4.63 (1.52)
9. Blood urea (m mol/L)	3.01 (0.93)	3.11 (0.71)	3.58 (0.84)	3.77 (1.16)	2.94 (0.26)

Mefloquine-artesunate combination gave the best cure rate of 98.27% compared to mefloquine (83.3%) and artesunate (75%). Fever and parasite clearances were significantly shorter in the two groups that contained artesunate than in the group that received mefloquine alone (p less than 0.002). However the parasite clearance time was more prolonged with the combination than with artesunate alone.

Artesunate as a single agent had the least side effects and the combination regimen had more frequent gastrointestinal symptoms and myalgia but required no treatment (Table 3). No cardiotoxic effects were observed in any group and the ECG's were within normal limits except for a single case of bradycardia (50 beats per minute) in the combination regimen. No haematological or biochemical adverse effects were noted on close follow up to 20 patients on the combination regimen (Table 4).

DISCUSSION

In uncomplicated falciparum malaria, the aim of drug treatment should be to achieve a cure with minimal toxicity. Artemisinin derivatives are now one of the most promising new drugs for malaria, because of their ability to reduce high levels of parasitaemia rapidly and with the least toxic effects; but their main drawback is the inability to achieve a cure because of high recrudescence rates.

Recrudescences usually indicate that the killing efficacy of a drug is low. Our present method of assessment i.e. looking at the peripheral blood smear has its limitations; because a negative blood smear does not necessarily mean that there are no more parasites in the patient's body. Subpotent levels undetectable by the conventional method may exist and

after a few erythrocytic cycles the increase in parasitaemia will recur, become detectable, and may later cause symptoms. Moreover, a reduction in killing efficacy is known to be a harbinger of drug resistance.

To achieve a complete clearance of parasites, recommendations have been made to supplement the artemisinin derivatives with another antimalarial drug for curative treatment [15].

Chinese scientists have carried out comparative studies on these combinations with other standard antimalarial drugs. A cure rate of 100% was reported in falciparum malaria patients receiving combination of i/m artemisinin plus oral sulphadoxine-pyrimethamine and primaquine [16]. They have also compared the following regimens:- (1) Mefloquine plus sulphadoxine-pyrimethamine, (2) mefloquine plus artemisinin, (3) artemisinin alone and (4) mefloquine plus sulphadoxine-pyrimethamine plus artemisinin on a total of 80 patients and found no recrudescences with regimens (1), (2) and (4) but observed 41% recrudescences in the patients receiving artemisinin alone [8]. Parasite clearance time was reported to be fastest with the artemisinin alone but slower on using artemisinin-mefloquine combination; and it was suggested that there might be some antagonism between the two drugs [17]. This is also observed in our present study (Table 2), but in our patients, the initial higher parasitaemia of the group receiving the mefloquine-artesunate combination probably accounted for the nonsignificant slight delay in parasite clearance.

Now that evidences of synergism between mefloquine and artemisinin derivatives are stronger [10-13] we thought it might be advantageous to administer them simultaneously in our trial.

An almost identical trial like ours carried out in Thailand between January and May 1991, but using the mefloquine-artesunate combination in sequence instead of simultaneously and supported by in vitro studies of representative isolates, has also reported a cure rate of 100% using the combination. The parasite and fever clearance times were also significantly better with the two regimens containing artesunate than with mefloquine alone [18]. Our results are comparable to those of Thailand.

Hence we conclude that the combination of sodium artesunate and mefloquine is more efficacious than the use of the drugs as single agents, to achieve a cure in uncomplicated falciparum malaria. However more extensive pharmacokinetic studies are still required before we can recommend the combination for general use.

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Clinical trial of antidiabetic traditional medicine formulation (TMF) No. 32

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Clinical trial to determine the therapeutic efficacy of a traditional antidiabetic drug, TMF-32, was carried out on six type II NIDDM patients who were admitted to the Traditional Medicine Hospital, Yangon. All patients, 3 males and 3 females, had a fasting blood glucose level of more than 150 mg% (205.2 ± 35.6) and a 2 hour post-prandial blood glucose level of more than 200 mg% (323.0 ± 62.09) and were clinically free from complications of diabetes. The study was a complete cross-over design, using tolbutamide as a control and was conducted under strict diabetic diet supplying approximately 2500 kJ per day. Hypoglycaemic efficacy calculated from oral glucose tolerance test (OGTT) curves indicated that TMF-32, at doses of 2G and 3G, significantly reduces the blood glucose levels in these patients ($p < 0.01$ in both doses). The maximum hypoglycaemic effect was seen at approximately 2 hours after dosing and the duration of action lasted only up to 6 hours. Construction of log. dose-response curves showed TMF-32 of having a hypoglycaemic potency of 73% of tolbutamide but frequent side effects and cost of drug give rise to contradicting questions on whether it may be useful as a standard traditional antidiabetic drug.

INTRODUCTION

In Myanmar, diabetes mellitus is not an uncommon disorder and antidiabetic traditional drugs have been used by many patients even though they have never been subjected to scientifically controlled trials in human patients.

Among the antidiabetic TMF's, TMF No. 32 has been recognised by many traditional physicians to be the most efficacious and previous *in-vivo* study in animal models (Aye Than *et al.*, 1991) as well as studies on human diabetic patients at the traditional medicine hospital (unpublished data) have shown it to be the most promising and adverse effects have not yet been reported.

This study was a preliminary trial, conducted with an attempt to find out the dose-response relationship of TMF-32, so as to establish its therapeutic status in diabetic patients.

PATIENTS AND METHODS

Subject selection

Six type II, non-insulin dependent diabetic patients, 3 males and 3 females, aged 57.3 ± 2.7 years, were selected according to the National Diabetic Data Group criteria (Singer *et al.*, 1989) for diabetes: (1) fasting blood glucose of more than 140 mg% on at least 2 occasions (2) random blood glucose of more than 200 mg%.

The patients were clinically free

from other diseases or complications of diabetes, and have not been taking any medication for the past 2 weeks. Pregnant and lactating mothers were also excluded from the study.

Six non-diabetic controls, 3 males and 3 females of comparable age (52.5 ± 5.5 yrs) were also selected. Thorough clinical examination was done on admission and daily recording of vital signs including pulse, blood pressure, respiration and body temperature as well as fluid intake and output monitoring and recording of drug toxicity was done throughout the study.

Study design

The trial was a randomized, 2-way cross-over design, with the hypoglycaemic efficacy of different doses of TMF No. 32 and tolbutamide evaluated on the same patient with a wash-out period of 24 hours between each dose of the same drug and 2 weeks between the individual drugs tested.

Eligible patients from the Traditional Medicine Hospital and dispensaries were selected according to the criteria set. They were randomly divided into two groups, Group 1 - TMF No. 32 and group 2 - tolbutamide. Only those patients who gave a voluntary informed consent were allowed to participate in the study. They were explained about the nature and purpose of the study and the right to withdraw at any stage without prejudice. All subjects were abstained from smoking and strenuous activity throughout the study period. The trial was conducted under strict diet control of approximately 2500 kcals per day, which was prepared and supplied at the Traditional Medicine Hospital.

After randomization, the patients were admitted to the Diabetic Unit at the TM hospital and a strict diet control instituted. Routine history

taking, clinical examination, lab investigations and random plasma glucose level was taken. The patient was fasted at 10 pm on day 2 followed by fasting plasma glucose estimation at 6 am and OGTT at 7 am on day 3. After a 24 hour wash-out period, the procedure was repeated with 1G, 2G and 3G TMF No. 32 and was discharged on day 10. The patient was readmitted after 2 weeks and then crossed-overed to a different drug, tolbutamide, and the whole procedure repeated.

The same procedure was also conducted on normal healthy volunteers with different doses of TMF No. 32.

Comparison of hypoglycaemic potency of the two drugs studied was done through comparison of the oral glucose tolerance test (OGTT) curves obtained by administration of 75 gram oral glucose and measuring the blood glucose levels at 30, 60, 120 and 240 minutes respectively. The blood glucose levels were determined by O-toluidine method.

RESULTS

A total of 12 volunteers, 6 diabetic patients and 6 normal controls aged between 40-60 years took part in the study. The diabetic patients had a fasting blood glucose level of 205.2 ± 35.6 mg% and a 2 hr post-prandial blood glucose level of 323.0 ± 62.1 mg%. The normal volunteers had a fasting blood glucose level of 101.0 ± 16.7 mg% and a 2-hour post-prandial blood glucose level of 110.7 ± 19.9 mg%.

Figure (1) & (2) showed the mean OGTT curves of the 6 patients studied, after different doses of tolbutamide and TMF No. 32. Table (1) showed the comparison of hypoglycaemic potency, given as reduction of area under the OGTT curves, between

Table 1. Relative hypoglycaemic potency of TMF No. 32 as compared with tolbutamide (plasma glucose AUC in gram % hours)

PATIENT	OGTT	TOLBUTAMIDE			T.M.F. No. 32		
		0.25 G	0.5 G	1 G	1 G	2 G	3 G
U.T.	1.535	1.800	1.755	1.385	1.325	1.250	1.165
U.S.M.	2.070	1.910	1.785	1.525	1.580	1.425	1.150
U.K.S.	2.985	2.910	2.840	2.610	2.775	2.430	2.490
D.T.S.	0.855	0.900	0.875	0.905	0.735	0.690	0.715
D.T.H.	2.980	2.710	2.490	1.860	2.790	2.260	2.450
D.T.N.	3.365	3.355	2.560	2.205	3.165	3.060	2.620
Total	13.790	13.585	12.305	10.490	12.370	11.115	10.590
Mean	2.298	2.264	2.051	1.748	2.061	1.853	1.765
SD $\pm$	0.979	0.895	0.723	0.609	0.979	0.878	0.845

tolbutamide and TMF No. 32. In the present study, tolbutamide 1G as well as TMF No. 32 in doses of 2G and 3G significantly reduced the OGTT curves of patients ($p < 0.05$) and can thus be taken as effective doses.

Figure 3 and Table 2 both showed the effect of TMF No. 32 on the blood glucose levels of normal healthy volunteers. No significant reduction

Table 2. Effect of TMF No. 32 on plasma glucose curves of non-diabetic controls (plasma glucose AUC in gram % hour)

Healthy volunteers	OGTT	1 G	2 G	3 G
N.L.	1.472	1.536	1.536	1.588
K.M.M.	1.592	1.648	1.614	1.636
D.T.A.	1.564	1.424	1.634	1.448
U.K.N.	1.648	2.040	2.040	1.638
K.M.S.	1.934	4.160	1.784	1.654
U.M.K.	1.382	1.828	1.704	1.602
TOTAL	9.592	12.638	10.312	9.566
MEAN	1.598	2.106	1.719	1.595
SD $\pm$	0.190	0.103	0.163	0.076

was seen in all doses. Point by point comparison of hypoglycaemic effect at different hours showed that Tolbutamide (1G) significantly reduced

the blood glucose levels at 1.5 hours ($p < 0.02$) and 2 hours ($p < 0.05$). While TMF No. 32 at 2G and 3G significantly reduced the blood glucose levels at 2 hour ($p < 0.01$) and 3 hours ($p < 0.05$) after drug administration. No significant reduction was seen with other doses.

Figure (4) showed the comparison of log dose-response curve of tolbutamide and TMF No. 32. Both drugs showed a good correlation between logarithm of dose and blood glucose reduction activity ($r = -0.9915$ for tolbutamide and $r = -0.9955$ for TMF No. 32 respectively), and TMF No. 32 was found to have a potency of 73% of tolbutamide.

TMF No. 32 - 3G causes adverse effects similar to hypoglycaemic symptoms such as dizziness, sweating, palpitations, nausea and breathlessness in 2 of the 6 patients and 1 of the 6 normal healthy volunteers studied. The symptom occurred within one hour after taking the drug and subsided gradually after half to one hour. But no such effects were seen with TMF No. 32 2G (or) less or with tolbutamide up to 1G.

DISCUSSION

Diabetes mellitus is a disorder of

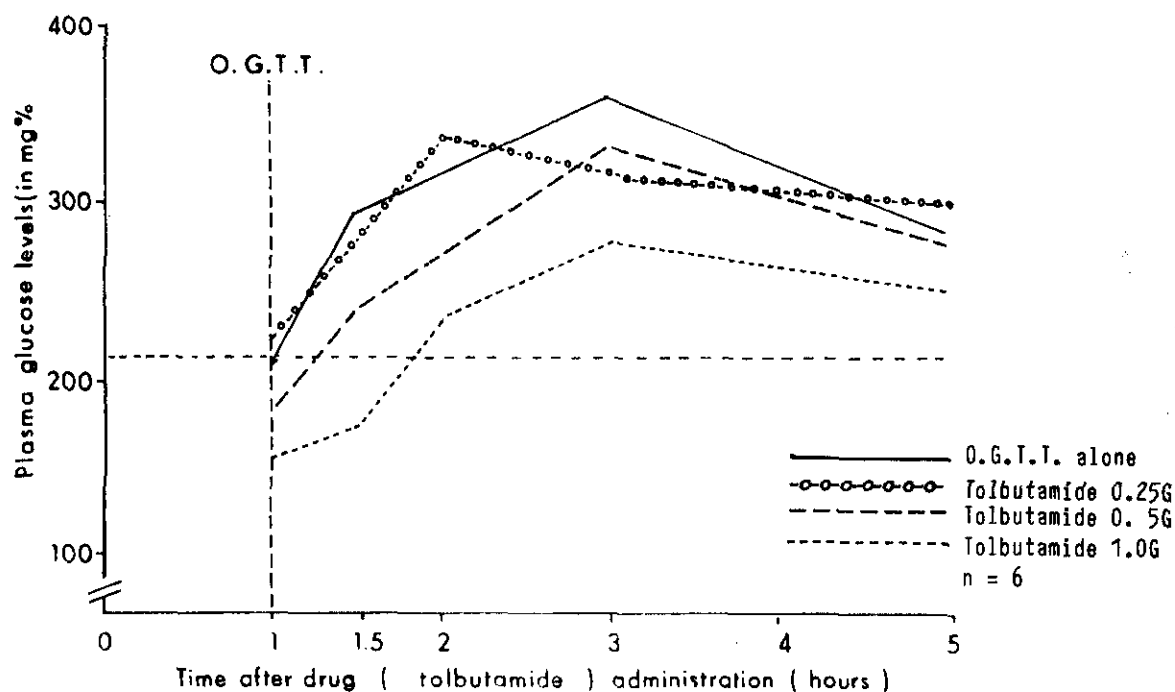


Figure 1. Oral glucose tolerance test (OGTT) curves of 6 diabetic patients after oral administration of different doses of tolbutamide (mean values).

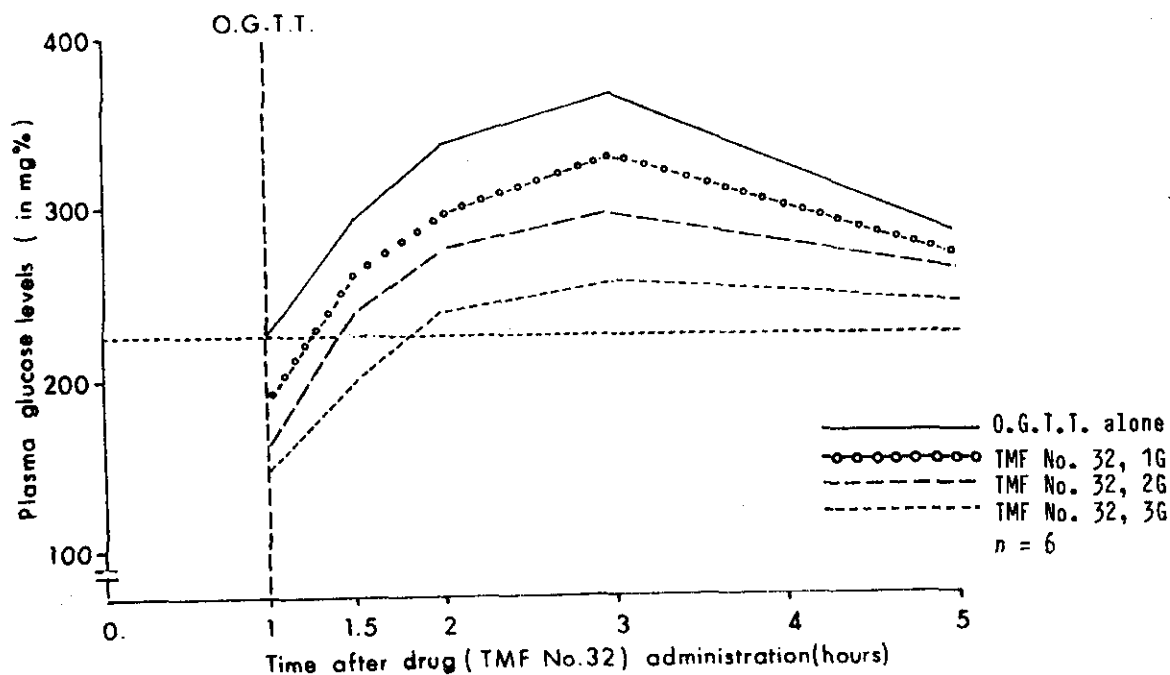


Table 2. Oral glucose tolerance test (OGTT) curves of 6 diabetic patients after oral administration of different doses of TMF No. 32 (mean values).

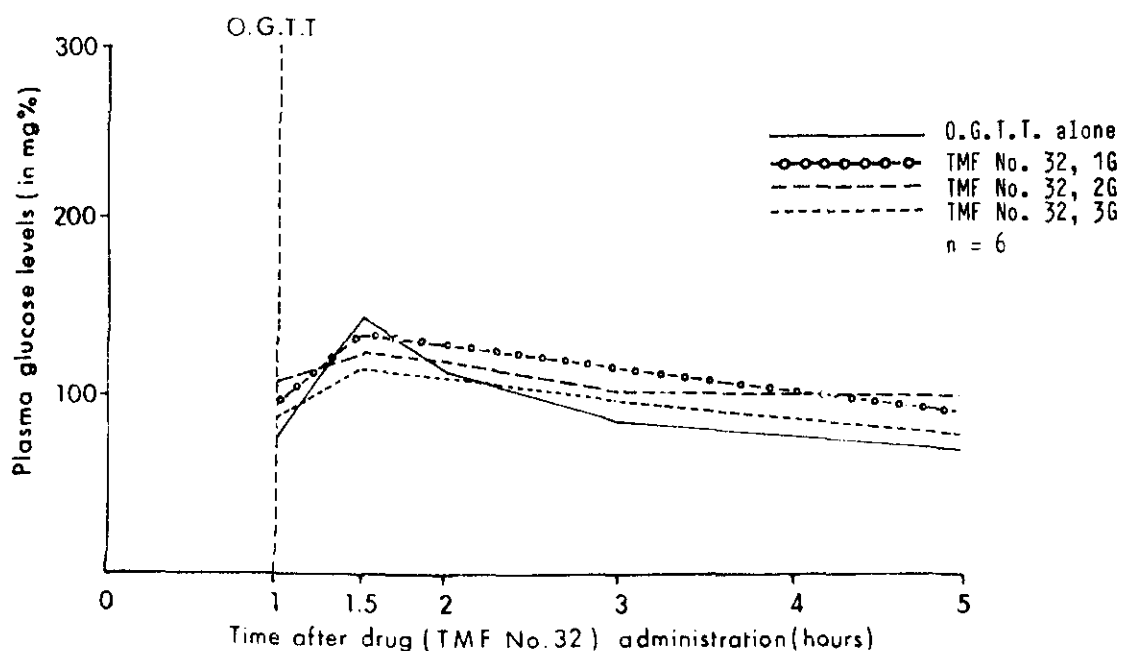


Figure 3. Oral glucose tolerance test (OGTT) curves of 6 healthy volunteers after oral administration of different doses of TMF No. 32 (mean values).

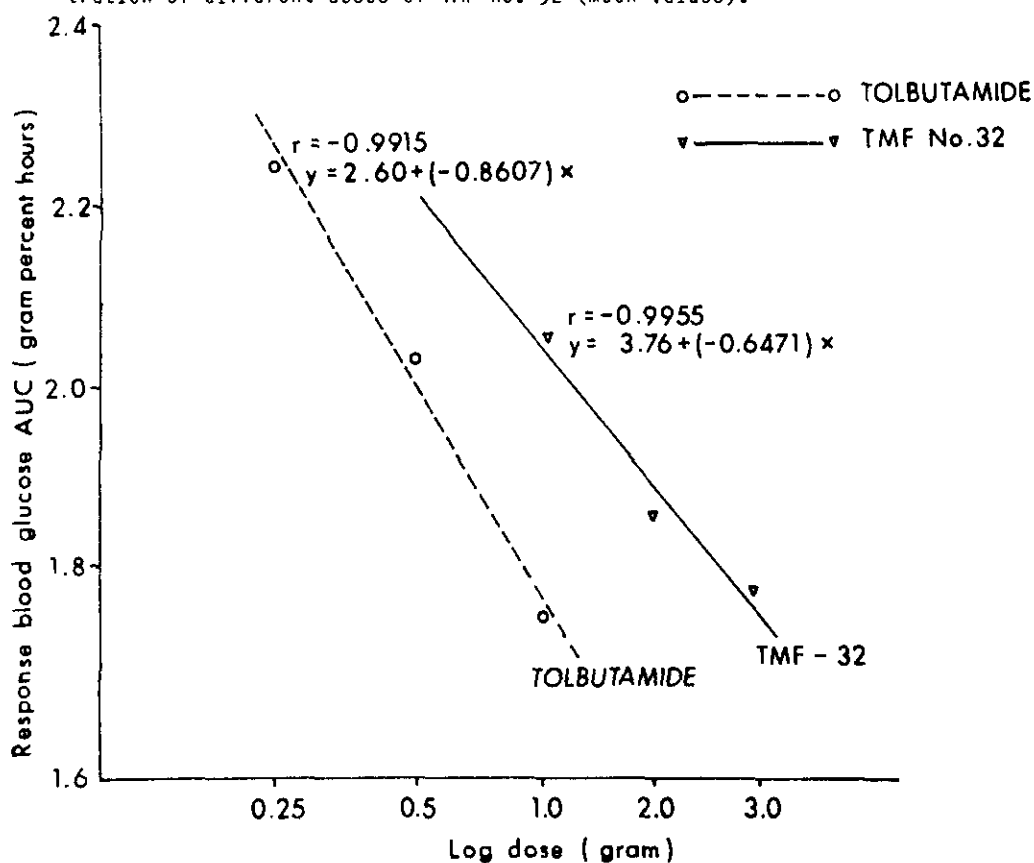


Table 4. Relative hypoglycaemic potency of TMF No. 32 compared with standard drug tolbutamide (TMF No. 32 is 73% as effective as tolbutamide)

carbohydrate metabolism characterized by elevated blood glucose concentration resulting from inefficient insulin secretion (Savage *et al.*, 1975), or peripheral and hepatic insulin resistance (Rizza *et al.*, 1981) or both. This chronic disorder, if left untreated, leads to development of ketoacidosis, neurovascular complications and ultimately increasing the morbidity and mortality of the patient (West *et al.*, 1980).

Effective treatment strategies in the management of diabetes includes; strict diet control, reduction in body weight, modification of life style (ie. cigarette smoking, physical inactivity) regulations of plasma lipids and blood pressure, etc., out of which, the maintenance of blood glucose levels to as near normal as possible is the most important. Especially in maturity onset type II, non-insulin dependent diabetes mellitus, oral hypoglycaemic agents occupy an important role in the control of blood glucose levels (Marchetti & Navalesi, 1989).

The main aim of all oral hypoglycaemic agents in the treatment of diabetes is of two fold (Marchetti & Navalesi, 1989).

- (1) The short-term aim is to control symptoms by restoring euglycaemia without major iatrogenic disturbances.
- (2) The long-term aim is to minimise the risk of neuro-vascular complications by maintaining:
 - (a) fasting blood glucose at or below 115 mg%
 - (b) two-hour postprandial blood glucose level at or below 144 mg%
 - (c) glycosylated haemoglobin (Hb A_{1c}) not higher than 9.0% (Dahl-Jorgensen, 1987).

The present study is focussed at the short-term aim, which is to find out

if TMF No. 32 is capable of restoring englycaemia without major iatrogenic disturbances.

Tolbutamide was chosen as a positive control for TMF No. 32 because previous studies on animal models (Aye Than *et al.*, 1991) and diabetic patients (unpublished data) had shown these two drugs to have similar onset and duration of action. Since the onset of action for both drugs is approximately 1 hour, OGTT was conducted 1 hour after drug administration.

The findings showed TMF No. 32 to be a short-acting hypoglycaemic agent having the potency of 73% of tolbutamide. The onset of hypoglycaemic action occurring at approximately 1 hour after drug administration and the maximal hypoglycaemic action occurring between 2 to 3 hours. The duration of action lasted only up to 6 hours.

In the present study, the effective dose for tolbutamide was 1G and for TMF No. 32, 2 to 3G respectively. But due to its adverse effects, TMF No. 32 should not be given in doses exceeding 2G. Although the adverse effects were every similar to hypoglycaemic symptoms, the blood glucose at the time of occurrence of symptom were found to persist well above the normal level of 80-120 mg%. This, may indicate that such effect can be due to other causes unrelated to hypoglycaemia.

TMF No. 32 has altogether 24 different contents of different origins (Table 3) (plants, animal, minerals etc.) and some by itself are considered toxic and some, very expensive and not easily available. Diabetes patients require diagnosed continuous treatment (many years, often life-long) and easily availability, low toxicity and cost, as well as good

Table 3. Ingredients of TMF-32 (Nandwin hsabut hsay gyee)

Scientific name	Family	Myanmar name	Part used	Amount in %
1. <i>Valeriana wallichii</i> DC.	Valerianaceae	Kantbalu	Rhizome	4.2
2. <i>Kaempferia</i> sp.	Zingiberaceae	Pan-oot	Rhizome	4.2
3. <i>Allium cepa</i> Linn.	Liliaceae	Kyetthunni	Rhizome	4.2
4. <i>Colocasia antiquorum</i> Schott.	Araceae	Peinmahawyar	Rhizome	4.2
5. <i>Gloriosa superba</i> Linn.	Liliaceae	Hsinmeedauk	Rhizome	4.2
6. <i>Allium sativum</i> Linn.	Liliaceae	Kyetthunbyu	Rhizome	4.2
7. <i>Polianthus tuberosa</i> Linn.	Amaryllidaceae	Hninpan	Rhizome	4.2
8. <i>Dioscorea</i> sp.	Dioscoreaceae	Kyway	Rhizome	4.2
9. <i>Cyperus</i> sp.	Cyperaceae	Myatmonhnyin	Rhizome	4.2
10. <i>Curcuma longa</i> Linn.	Zingiberaceae	Nanwin	Rhizome	4.2
11. Unidentified		Tawkhatta	Rhizome	4.2
12. <i>Amorphophallus campanulatus</i> Blume	Araceae	Wa	Rhizome	4.2
13. <i>Crinum asiaticum</i> Linn.	Amaryllidaceae	Koyankyee	Rhizome	4.2
14. <i>Antidesma</i> sp.	Euphorbiaceae	Kinpalin	Root	4.2
15. <i>Datura fastuosa</i> Linn.	Solanaceae	Padaingnet	Root	4.2
16. Unidentified		Gyatnet	Root	4.2
17. <i>Acacia farnesiana</i> Willd.	Mimosaceae	Nanlonkyaing	Root	4.2
18. <i>Emblica officinale</i> Gaertn.	Euphorbiaceae	Zibyu	Root	4.2
19. Unidentified	Compositae	Pannyo	Leaf	4.2
20. <i>Pogostemon patchouli</i> Pell.	Labiatae	Tanatpyitsee	Leaf	4.2
21. Unidentified		Yaungbyuhsay	Leaf	4.2
22. Calcium oxide		Maphaukhton		4.2
23. <i>Antiaris toxicaria</i> (Pers) Lesch.	Moraceae	Hmyaseik	Latex	4.2
24. <i>Moschus moschiferus</i> Linn.	Cervidae	Kadde	Dried secretion from the preputial follicles of the musk deer	4.2

San-Khin (9), Hundley and Chit-Ko-Ko (10)

compliance play a very important role in achieving successful anti-diabetic therapy.

Thus, further research and refinement such as search for active principle, exclusion of toxic and inactive components or finding a more easily available or less expensive alternative ingredients may need to be done if TMF No. 32 is to become a useful standard traditional antidiabetic drug.

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Isolation of haemolytic Escherichia coli from patients with urinary tract infection

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One hundred and eighty-five isolates of Escherichia coli isolated from different sources, such as 131 isolates from 58 diarrhoea cases, 33 isolates from 21 control cases and 21 isolates from 9 urinary tract infection cases with different age groups were studied for haemolytic activity. It was observed that one case from diarrhoea cases showed alpha haemolytic activity; none of the control cases showed any haemolytic activity; and 3 cases of urinary tract infection showed beta haemolytic activity. The haemolytic E. coli isolates were also performed for the plasmid encoded haemolysin determinant and demonstrated to possess the haemolytic phenotype Hly and encodes 107,000 kilodalton. E. coli isolated from the urinary tract infections were also tested for antibiotic sensitivity and found that most of them were resistant to ampicillin, streptomycin and tetracycline.

INTRODUCTION

Some of the Escherichia coli strains in humans secrete a cytotoxin, designated as E. coli hemolysin, that produces the characteristic zones of beta-hemolysis surrounding bacterial colonies on blood agar was reported by many investigators [1-6]. Studies using genetically engineered E. coli strains have demonstrated that E. coli hemolysin significantly contributes to bacterial virulence in animal models [7-10]. Antibodies to E. coli hemolysin are present in plasma of all adults, indicating that the toxin is produced by bacteria in human hosts [11-12]. It is generally assumed that E. coli hemolysin similarly represents a virulence factor of E. coli in humans.

MATERIALS AND METHODS

This study includes 185 isolates of E. coli isolated from different cases, such as 131 isolates from 58 diarrhoea cases and 33 isolates from 21 control

cases and more over 21 isolates of E. coli from 9 cases of urinary tract infection (UTI), with different age groups including children and adults.

For identification of organisms isolated from diarrhoea it was processed as according to the method of WHO [13] and Tin Aye *et al.*, 1984 [14]. For organisms from urinary tract infections it was followed as described by Tu *et al.* [15]. Selection of E. coli was done as in conventional methods used for screening of E. coli on Mac-Conkey agar (Nissui) for both diarrhoea and urinary tract infection cases. Identification of organisms was done by biochemical and with available 24 antisera for serological test. For haemolytic activity colonies were streaked onto 5 percent sheep blood agar and kept at 37°C overnight. Haemolysis (beta) was recorded as a clear colorless zone around the E. coli colonies, in which the erythrocytes have undergone complete discoloration. In some

cases an indistinct zone of partial destruction of erythrocytes about the colony, often accompanied by a greenish to brownish discoloration of the medium appeared (alpha haemolysis). Antibiotic sensitivity pattern was done by using Muller Hinton agar with its respective broth as described by Beuer et al., 1966 [16]. The antibiotic sensitivity discs used were chloramphenicol 100 mcg, kanamycin 50 mcg, streptomycin 50 mcg, and tetracycline 200 mcg which were supplied from JICA. Ampicillin 2 mcg (BBL), gentamycin 10 mcg (BBL); Trimethoprin sulfamethoxazole 23.75 mcg (BBL) and Urospasmon (Ludwig) which contains nitrofurantoin 100 mcg and sulfadiazine 100 mcg were also supplemented. The haemolytic *E. coli* isolated were also performed for the plasmid encoded haemolysin determinant and compared with the controls. Small scale preparations of plasmid DNA was performed as described by Sambrook et al., 1989 [17] with the modifications of Birnboim and Doly, 1979 [18] and Ish-Horowitz and Burke, 1981 [19].

RESULTS

131 isolates of *E. coli* from 58 cases of diarrhoea and 33 isolates from 21 control cases and found that only one case from diarrhoea produced an alpha haemolysis on blood agar. When tested with 21 isolates of *E. coli* from 9 urinary tract infection (UTI), 3 cases (9 *E. coli*) produced beta haemolysis on blood agar which also include one child. All these 9 cases of urinary tract infection produced significant growth of *E. coli*. From these patients, *E. coli* were also tested for serological activity with the available antisera but non of them showed any serological reactions. The antibiotic sensitivity pattern was shown in Table 1. It was found that all 9 patients were

Table 1. Sensitivity to chemotherapeutic agents of *E. coli* isolated from significant bacteriurics

		Japan (8mm) BBL (6mm) (6mm)								
Patients	Sex/Age	C	K	S	T	A	Gm	SxT	U	
1 h	F 5	34	23	-	-	-	20	-	15	
2 h	F 35	35	25	25	30	-	22	20	16	
3 h	F 50	22	21	22	22	10	20	24	16	
4	F 42	27	20	16	22	10	18	22	20	
5	F 27	30	30	-	30	25	30	26	22	
6	F 62	10	20	-	-	-	22	20	16	
7	F 36	-	-	-	-	-	20	18	18	
8	M 40	30	24	15	15	15	22	28	15	
9	M 76	25	20	-	10	-	22	22	15	

C = Chloramphenicol (100 mcg/disc)

K = Kanamycin (50 mcg/disc)

S = Streptomycin (50 mcg/disc)

T = Tetracycline (200 mcg/disc)

h = haemolytic *E. coli*

A = Ampicillin (2 mcg/disc)

Gm = Gentamycin (10 mcg/disc)

SxT = Trimethoprim (1.25 mcg) + Sulfamethoxazole (23.75 mcg)

U = Urospasmon (Nitrofurantoin 100 mcg + Sulfadiazin 100 mcg)

shown to be sensitive to Gentamycin and Urospasmon. Eight out of 9 patients showed a sensitive pattern to chloramphenicol, kanamycin and septrin. It was also observed that 4, 6 and 4 patients out of 9 patients tested were sensitive to streptomycin, tetracycline and ampicillin respectively.

Figure 1 also represent the plasmids of haemolytic *E. coli* isolated from bacteriurics and compared with the controls. It was shown by comparing with the controls the specific genetic information for the hemolytic phenotype hly A encodes a 107,000 kilodalton protein, which seems to be an inactive precursor of haemolysin.

DISCUSSION

Haemolytic (Hly⁺) strains of *Escheri-*

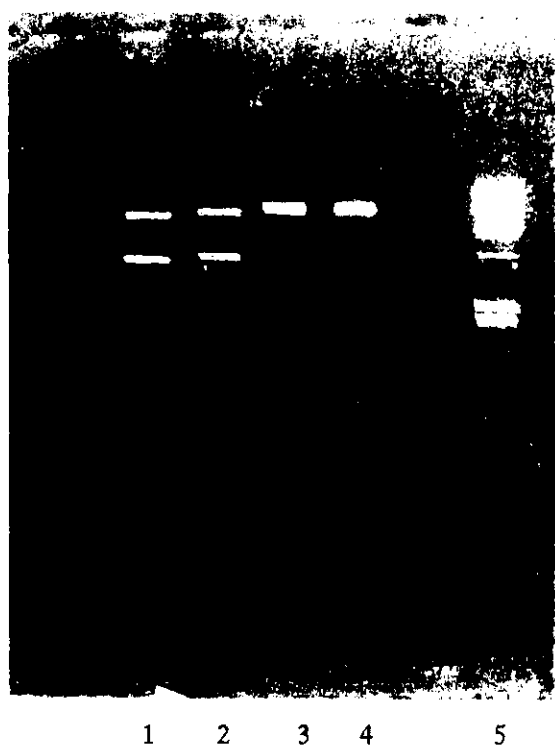


Fig. 1. EcoRI restriction endonuclease digestion pattern of plasmid DNA from haemolytic *Escherichia coli* isolated from urinary tract infection

1. *E. coli* from patient 3
2. *E. coli* from patient 3
3. *E. coli* from patient 1
4. Known haemolytic *E. coli* (Hayashi)
5. Lambda DNA digested with Hind III

Escherichia coli may be isolated from a number of sources. Those of animal intestinal origin [20] have often been found to carry the Hly determinant on transmissible plasmids [21-24] and Goebel and Hedgpeth [25] have shown that Hly plasmids of different origins and in compatibility groups carry the same Hly determinant [26]. Among humans, Hly⁺ *E. coli* occur only infrequently in the normal fecal flora but are often associated with extraintestinal infections such as

urinary tract infection and bacteremia [1,2,4]. These strains generally carry the Hly determinant on the chromosome [6] and recent studies indicate that chromosomal and plasmid Hly determinants, although not completely identical share a high degree of homology.

The wealth of available molecular genetic data on *E. coli* hemolysin stands in contrast to the paucity of knowledge regarding its mechanism to cytotoxic action. Among the major medically relevant bacterial cytotoxins, *E. coli* haemolysin is certainly one of the poorest characterized in terms of functions. Eberspacher *et al.*, 1989 [27] reported the quantitative study of the binding and hemolytic efficiency of *E. coli* hemolysin and their data support the pure concept of toxin action by showing that attachment of a low and finite number of toxin molecules to an erythrocyte will ultimately generate a cytolytic lesion.

Noegel *et al.* (1981) [28] previously provided evidence based on exclusion chromatography that the *E. coli* hemolysin protein was ca 120 kilodaltons. Mackman and Holland [29] showed that a 107 kd polypeptide, the putative hemolysin precursor of the Goebel model, was efficiently released from hemolytic *E. coli*. These investigators provided indirect evidence that this polypeptide was the active hemolysin encoded by Hly A cistron [25].

Goebel and Hedgpeth [25] also showed 4 factors as: (i) At least three cistrons hly C, hly A and hly A contain the specific genetic information for the haemolytic phenotype. (ii) Hly A encodes a 107,000-kilodalton protein, which seems to be an inactive precursor of hemolysin. (iii) Normal amounts of hemolysin activity requires

only the products of hly A and hly C and this activity was found in the periplasm; very little hemolysin activity was found in the cytoplasm, suggesting that the hly C product is required for transport or activation of the hly A product or both. (iv) Active haemolysin remains in the periplasm in the absence of hly B function, hence the hly B product seems to be necessary for the transport of haemolysin to the exterior of the cell. Over-production of the hly A product is lethal, probably causing lysis of the cell.

From our previous study by Tu et al. [15] 6.2 percent of the population of the rural group and 4.2 percent of the urban group the organisms isolated were Streptococcus faecalis 49.5 percent, Escherichia coli 43.2 percent, Staphylococcus aureus 9.5 percent, Proteus sp. 12.6 percent, Enterobacter aerogenes, 3.2 percent and Klebsiella aerogenes 6.3 percent and out of isolates of E. coli 2 of each belonged to 0119/B14 and 0128 B12 serotypes. In our study only E. coli was isolated with significant growth with unknown serotypes. It was interesting to note that 46.6 percent, 34.2 percent, 24.5 percent, 12.2 percent, and 29.3 percent showed resistant to ampicillin, chloramphenicol, gentamycin, kanamycin and tetracycline. In our study 5,5,3 cases out of 9 cases tested were resistant to ampicillin, streptomycin and tetracycline respectively. Two cases was resistant to chloramphenicol and one case each to kanamycin and streptomycin. When checking with patients it was found that 3 patients were shown to be sensitive to all the antibiotics tested. The other patients were resistant to one to five antibiotics. 2 cases were resistant to ampicillin.

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**Intestinal permeability in Myanmar children:
Results from a field study**

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The differential urinary excretion of lactulose and L-rhamnose after a standard oral dose has been employed as an index of mucosal injury and its feasibility and practicability tested under field conditions. 110 children who were assessed for malnutrition also had intestinal permeability analysis. Weight-for-age were all used as indicators of malnutrition. Moderately malnourished children (75% weight-for-age) had significantly greater lactulose/L-rhamnose ratios ($p < 0.05$) than those children with normal nutrition (90% weight-for-age) (0.1495 ± 0.0134 vs 0.1132 ± 0.0109). No significant differences were found between the differential sugar ratios of those children who had chronic (90% length-for-age) or acute (80% weight-for-height) malnutrition and those children who were normally nourished according to these classifications.

INTRODUCTION

Malnutrition and infection are common findings in children in developing countries and constitute one of the world's dominant public health problems. Estimates of the prevalence of malnutrition in developing countries vary according to socioeconomic status, region, season, age and sex: it may range from 30 to 90 per cent, with severe malnutrition ranging from one percent to ten percent [1]. However, a greater majority of children in the underprivileged environment suffer from borderline malnutrition and are at constant risk of sliding into manifest malnutrition [2]. Of all the illnesses in developing countries, enteric infections account for up to half; an estimated one billion episodes and five million deaths occur globally every year in under five children [3].

It is generally accepted that a reciprocal relationship exists between malnutrition and infection. Malnutrition has been shown to increase the

duration of infections [4-6], and increase the number of subsequent complications [7], and morbidity and mortality rates have been shown to be higher in the malnourished [8].

The effects of infection on nutritional status through excessive metabolic losses and decreased food intake are well documented [9-11]. Of all the infectious diseases, enteric infection has the greatest adverse effect on growth [12].

To these effects of clinically apparent infectious disease on the hosts' nutrition must now be added the potential effects of a chronic, subclinical, enteric disorder [13,14]. This widespread, usually asymptomatic "sub-clinical enteropathy" has been demonstrated by morphological abnormalities in virtually the entire low income adult population in developing nations compared with normal adults from the West. Similar changes are found in the small intestine of the children in these countries. Evidence for existence of subclinical enteropathy

length for age indicates chronic malnutrition (stunting).

Intestinal permeability analysis

On the morning of the test, parents were instructed to give an early breakfast to the children to be tested. In addition, they were requested to provide a morning urine sample which was collected in sterile urine container to which 2 drops of 10% thiomersal was added. In a field study, it was impractical to ask children to fast overnight. The basal urine sample was necessary as a precaution to detect any basal levels of lactulose present in the urine before the test commenced.

Three to four hours after they had eaten breakfast, they were given a 100 ml each of isotonic solution containing 5g lactulose (7.5ml Duphalac) and 1g L-rhamnose in water. The solution was coloured to make it more attractive. For the first hour of urine collection, the children were not allowed to eat or drink. After this, drinking of water was encouraged. In the first fifteen minutes of five hours, all children were encouraged to void all urine. A 5 hour urine collection was made into a plastic bottle containing 10 drops of 10% solution of thiomersal. The 5 hour urine volumes were recorded and aliquots of the 5 hour and the basal samples were taken and stored at -20°C until the time of analysis.

Urinary lactulose and rhamnose were carried out within 2 months after collection of samples. Urinary lactulose and L-rhamnose were estimated by modification of quantitative thin layer chromatography [18]. Briefly, sugar separation was achieved by multiple development on half-plate (10 cm - 20 cm) of glassbacked silica gel 60 (E. Merck, Darmstadt,

FRG) using two consecutive ascending runs (8.5 cm each) with butan-1-ol/ethanol/acetic acid/water (60:30:10:10, by vol). The layers were dried for at least 30 min between each run, and then 4 hour (preferably overnight) to remove the solvent before performing a 4-aminobenzoic/phosphoric acid colour reaction at $120-130^{\circ}\text{C}$ for 10 min [19]. The separated zones were measured with a recording and integrating densitometer (Quick Scan Jr. TLC, Helena Laboratories, Beaumont, Texas). Sugar concentrations were calculated by comparing the test with standard peak areas.

RESULTS

Malnutrition and its association with intestinal permeability was assessed in 110 children. The lactulose/L-rhamnose ratios were found to be significantly higher ($p < 0.05$) in those children who were moderately malnourished (75% weight for age) (mean 0.1495; range 0.029-0.5707) compared to those children who were nourished (greater than or equal to 90% weight for age) (mean 0.1132; range 0.06-0.1965). No significant differences in the sugar ratios were found between moderately malnourished and mildly malnourished children ($p > 0.05$) (Table 2).

Children were also grouped according to length for age as an indicator of chronic malnutrition. No significant differences were found when the lactulose/L-rhamnose ratios of the normally nourished (mean: 0.1315; range: 0.039-0.436), mildly malnourished (mean: 0.1438; range: 0.029-0.4045) and moderately malnourished (mean: 0.1599; range: 0.034-0.5707) were compared with each other ($p > 0.05$ in each comparison) (Table 3).

Sugar ratios determined for the

has been found in studies done in the developing areas of the world [13].

Few tests are available which both assess mucosal function and which are suitable for use in the field. Most of the procedures available tend to be invasive, similar to intestinal perfusion and biopsy or, like the D-xylose absorption test, to be affected by many irrelevant variables. A new method has been established based on the excretion of test substances following ingestion. The differential urinary excretion of these substances after ingestion has been employed as an index of mucosal damage, the urinary sugar excretion ratios (e.g. lactulose/L-rhamnose) rising in the presence of villous atrophy.

The present study was carried out with the objectives of determining the feasibility and applicability of differential sugar absorption test in children under field conditions and to determine intestinal permeability in children with and without malnutrition.

SUBJECTS AND METHODS

The study population comprised of 110 children aged 31-56 months; 57 were males and 59 females. All the children lived in North Okkalapa, peri-urban area, in Yangon. The present study was conducted during the months of July and August 1988. In the first visit, children were weighed and measured so that nutritional status could be determined. Urine collection for assessment of intestinal permeability was done on the second and third visits.

Assessment of malnutrition

On the first week of the field study, sex, age in month and height of all children were recorded. All instru-

ments used were checked and calibrated before use in the field. Weights were measured using bathroom scales (SECO) sensitive to 0.25 kg. Children were weighed with minimal clothing on (cotton dress for the girls; cotton shorts and shirts for the boys). Supine lengths of the children were measured using an infantometer made of teak and non-stretch plastic tape. Length was measured to the nearest 0.5 cm. Weights and heights were measured in accordance with WHO guidelines [16]. Growth curves of the National Center for Health Statistics were used as the reference [17]. Percentages of the median values of these growth curves were used as definitions for categories of weight for age, weight for height and length for age (Table 1).

Table 1. Classification of nutritional status of children using percentages of the median values of growth curves of the national center for health statistics.

Nutritional status	Percent of standard
Weight for age	
Normal	> 90%
Mild	75-89%
Moderate	60-74%
Severe	< 60%
Length for age	
Normal	> 95%
Mild	90-94%
Moderate	< 90%
Weight for length	
Normal	> 90%
Mild	80-89%
Moderate	< 80%

All 3 measurements of malnutrition were used, as the single anthropometric measurement of weight for age does not distinguish between acute and chronic malnutrition. A low weight for height indicates current malnutrition while a low

Table 2. Intestinal permeability and malnutrition (weight for age)

Nutritional status (Weight for age)		Intestinal permeability (Lactulose/L-rhamnose) (Mean $\pm$ SD)
Well nourished (Weight for age >90%)	X_1	$.1132 \pm 0.0109$ (n = 13)
Mild malnourished (Weight for age 75-89%)	X_2	0.1364 ± 0.0129 (n = 14)
Moderate malnourished (Weight for age 60-74%)	X_3	0.1495 ± 0.0134 (n = 57)

Test of means

X_1 vs X_2	t = 1.374	p > 0.05
X_2 vs X_3	t = 0.7043	p > 0.05
X_1 vs X_3	t = 2.1015	p < 0.05

Table 3. Intestinal permeability and malnutrition (length for age)

Nutritional status (Length for age)		Intestinal permeability (Lactulose/L-rhamnose) (Mean $\pm$ SD)
Well nourished	X_1	0.1315 ± 0.0107 (n = 49)
Mild malnourished	X_2	0.1438 ± 0.0134 (n = 44)
Moderate malnourished	X_3	0.1600 ± 0.0308 (n = 17)

Test of means

X_1 vs X_2	t = 0.717	p > 0.05
X_2 vs X_3	t = 0.8710	p > 0.05
X_1 vs X_3	t = 0.479	p > 0.05

groups of normally nourished, mildly and moderately malnourished children according to their weight for length were shown in Table IV. The mean lactulose/L-rhamnose ratio of the mildly malnourished children (90-94.9%

Table 4. Intestinal permeability and malnutrition (weight for length)

Nutritional status (Weight for length)		Intestinal permeability (Lactulose/L-rhamnose) (Mean $\pm$ SD)
Well nourished (Weight for length 90%)	X_1	0.1333 ± 0.01 (n = 32)
Mild malnourished (Weight for length 80-89%)	X_2	0.1518 ± 0.0164 (n = 49)
Moderate malnourished (Weight for length 80%)	X_3	0.1306 ± 0.0133 (n = 29)

Test of means

X_1 vs X_2	t = 0.963	p > 0.05
X_2 vs X_3	t = 0.16	p > 0.05
X_1 vs X_3	t = 1.0	p > 0.05

weight for length) was higher (mean: 0.1518; range: 0.034-0.436) than the mean ratio for the moderately malnourished (mean: 0.1306; range: 0.0341-0.249). The mean ratio for the normally nourished children (mean: 0.1333; range: 0.0375-0.2475) was also marginally higher than the mean ratio for the moderately malnourished children. However, no significant differences between the lactulose/L-rhamnose ratios were found when each group was compared (p > 0.05).

DISCUSSION

Our study documents the fact that differential sugar absorption test in children is feasible and applicable under field conditions. As it was impractical to ask children to fast overnight, basal urine samples were determined for basal levels of lactulose. The children were able to tolerate 100 ml of test solution, and 5 hour urine collection was also

feasible.

As a group those children with moderate malnutrition have increased lactulose/L-rhamnose excretion ratios compared to those children who are normally nourished. This excretion ratio is a sensitive indicator of mucosal damage [20], and the difference suggests that the moderately malnourished children have a more damaged small bowel mucosa than the normally nourished children. A few past studies done on malnutrition and intestinal permeability have focussed primarily on severely malnourished children who have been noted to have a greatly increased lactulose/mannitol ratio (3 times) compared to normally nourished children [21], and decreased transport of PEGs which improved slowly with nutritional rehabilitation [22]. In a study [19] carried out on moderately malnourished children looked at the permeability characteristics of children between 60 and 80% weight for age. The study was done on infants and revealed that these children had lactulose/mannitol ratios just significantly greater than those for normally nourished infants. No other workers as yet have looked at the lactulose/L-rhamnose ratios in children with mild (75-89% weight-for-age) and moderate malnutrition (60-80% weight-for-age). The results, however, seem to correlate with a recent study by Sepulveda *et al.* [1] where children with moderate malnutrition was found to experience a risk of diarrhoea approximately 60 percent greater than those with normal nutrition. This increased risk may be associated with a more damaged mucosa in the moderately malnourished children.

If malnutrition does not play a direct role in the genesis of the mucosal damage, some other factor must be responsible for both the mucosal

alterations and malnutrition. As this factor seems also to be related to the contaminated environment, the most likely cause for both is bacteria.

Although this study has not made direct comparisons between child populations in developing and Western countries, "normal" sugar ratio values for healthy adults and children in Western countries have been established and although not scientifically satisfactory, rudimentary comparisons can be made between these values and those from the present study. Further work would need to be done in this area to follow up these findings.

If the sugar ratios of the whole healthy population studied seem to indicate that all the Myanmar children studied have increased mucosal damage compared to those children in Western nations, the cause of this damage needs to be clarified. It is most probably due to the widespread sub-clinical enteropathy noted in virtually the whole apparently healthy population in most developing countries. It is evident that further work needs to be done in this area and the understanding can then be directed towards the management of malnutrition and infection in children in under developed countries.

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Identification of cultivated Chounggyah Plant and comparative pharmacognostic studies with market sample

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Chounggyah plant cultivated in the herbal garden of Traditional Medicine Hospital, Mandalay was identified to be Vernonia elaeagnifolia DC. Comparative pharmacognostical, physicochemical and phytochemical studies revealed that cultivated and market Chounggyah are not identical.

INTRODUCTION

Chounggyah market sample is being used as one of the ingredients in the Traditional Medicine Formulations (TMFs). This stem is also used individually as thirst quencher, blood purifier, antipyretic and also as galactagogue. Due to the above qualities the stem is used as one of the ingredients in TMF-16 Apunyein-thway-zay [1]. So far, the scientific name of this crude drug has not yet been identified. In order to reveal the scientific name, complete reproductive plant parts are required. Therefore a climbing plant "Chounggyah", from the herbal garden of Traditional Medicine Hospital, Mandalay was used for identification of the scientific name of the market Chounggyah.

In the present investigation the scientific name of the cultivated Chounggyah was identified and the stem of the market sample and that of the identified plant was studied for comparison to determine their identity in their pharmacognostical, physicochemical and phytochemical characters.

MATERIALS AND METHOD

Material

The plant used for identification in this study was obtained from the herbal garden of Traditional Medicine Hospital, Mandalay. Sample of drug (stem) sold under the name of "Chounggyah" was purchased from the local drug stores. A voucher specimens representing the collections are deposited in the herbarium and the crude drug museum of the Pharmacology Research Division, Department of Medical Research, Yangon.

Method

1. Taxonomical and pharmacognostical identification

Identification of the cultivated plant was done by reference to descriptions given by Hooker [2] and Brandis [3]. Permanent stem sections were made following the usual plant microtechnique Johansen [4] and Gray [5]. Ultra-violet fluorescence analysis of stem powders and the extracts of the stems in different solvents were carried out according to the method

of Chase and Pratt [6]. The ash values were determined according to the methods of Pharmacopoeia of India [7].

2. Physicochemical and phytochemical characterisation

The air dried stem powders were successively extracted with petroleum ether (60-80°C), chloroform, alcohol and the aqueous extracts were used for phytochemical analysis according to standard phytochemical method and thin layer chromatography was conducted with the chloroform and ethanolic extract according to physicochemical standards of Unani formulations [8].

RESULTS

Morphology

A climbing plant with, long finely strated branches, alternate linear-oblong or elliptic-lanceolate leaves; hoary inflorescence consisting of 3- to 5- flowered heads in narrow terminal, short loose panicles; fruit with white persistant pappus was identified to be Vernonia elaeaginifolia DC. belonging to family Compositae (Fig. 1).



Fig. 1. Flowering branch of "Chounggyah" (Vernonia elaeaginifolia DC.)

MACROSCOPIC FEATURES OF THE STEM

Vernonia elaeaginifolia DC. "Chounggyah" (Fig. 2)

Stem cylindrical, externally ash-grey with finely longitudinal striations, puberulous. Internally light brown, with spongy pith.

Chounggyah Market Sample (Fig. 2)

Stem cylindrical, fibrous, externally dark brown with longitudinal striations, glabrous. Internal cut surface light brown with solid pith.

MICROSCOPIC CHARACTERS OF THE STEM

Vernonia elaeaginifolia DC. (Fig. 3a)

The transverse section of the matured stem shows the following characters: epidermis consists of a single layer of epidermal cells followed by 12 to 14 rows of irregular thin wall cortical parenchyma cells. Groups of sclerenchyma cells are scattered in patches in the cortical region. Below the cortical region lies the phloem where sclereids in groups of two's and three's are imbeded. Xylem region is a broad zone consisting of numerous large trachea and thick walled xylem parenchyma. Pith is a central zone consisting of two types of parenchyma; irregularly shaped parenchyma followed by isodiametric parenchymatous region, where patches of sclerenchyma cells are seen.

"Chounggyah" market sample (Fig. 3b)

The following characters are found in transverse section of the stem. Periderm region consists of 8-10 layers of thin wall irregular flattened compactly arranged cortical parenchyma cells. Tanniferous cells are present throughout this region.

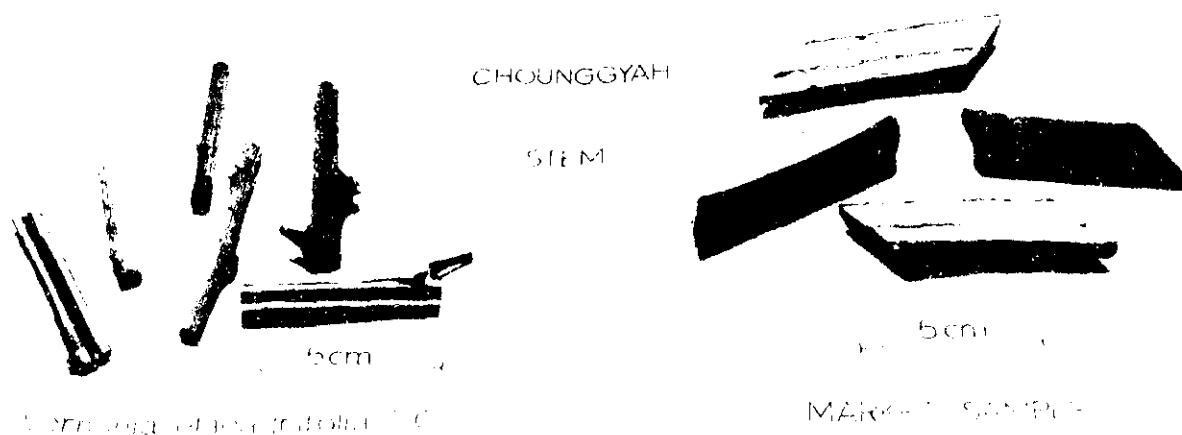


Fig. 2. Macroscopic features of Chounggyah stems

Phloem fibres are present in the form of conical patches separated by rectangular flattened thin walled parenchyma cells. Secondary xylem is a broad zone of numerous radially arranged xylem separated by medullary rays. Pith is a central zone of parenchyma region consisting of isodiametric thin wall parenchyma.

FLUORESCENCE ANALYSIS

The powders of the stem of *Vernonia elaeagnifolia* DC. and market Chounggyah and the extracts in different solvents were examined under visible and UV light. The stem powders were treated with various reagents as described as recorded in Table 1.

PHYSICOCHEMICAL ANALYSIS

The average values of loss of weight on drying at 105°C, total ash, acid-insoluble, water-soluble ash and extractive values are presented in Table 2.

PHYTOCHEMICAL ANALYSIS

The extracts from various solvents were tested for alkaloids, carbohydrates, fixed oils, resinified volatile oils, flavonoids, glycosides, phenols,

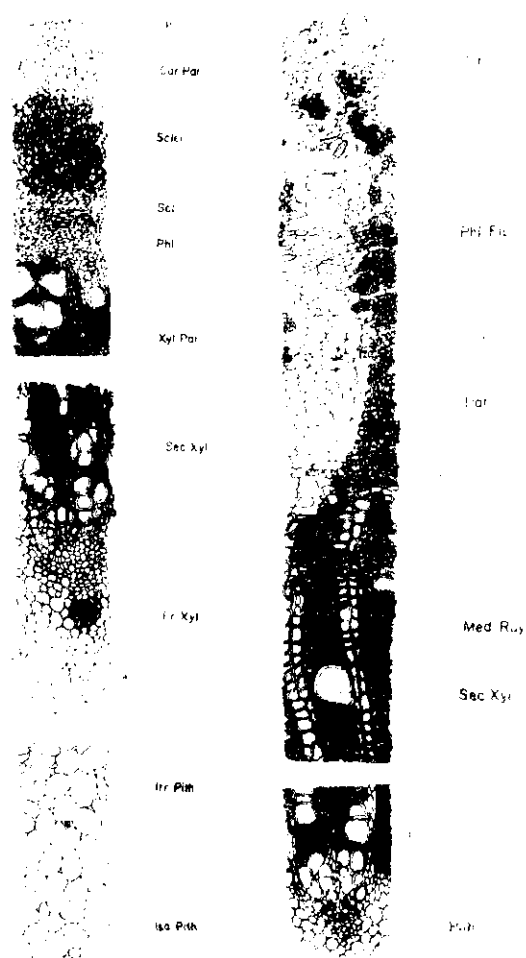


Fig. 3. T.S. of Chounggyah Stems

- a. *Vernonia elaeagnifolia* DC.
- b. Market Chounggyah

Table 1. Fluorescence characteristic of stem powders Vernonia elaeagnifolia DC. and market Chounggyah

Treatment	<u>Vernonia elaeagnifolia</u> DC.		<u>Market Chounggyah</u>	
	Visible light	UV light	Visible light	UV light
Powder as such	Yellow	Pale bluish grey	Pinkish yellow	Pale ash grey
Powder + 1N NaOH (aqueous)	Light brown	Brown with orange tinge	Dark brown	Brown at the centre green at the edge
Powder + 1N NaOH (methanol)	Yellow brown	Lemon yellow	Brown	Light greenish blue
Powder + 1N HCl	Light yellow brown	Light beige brown	Brown with orange tinge	Brown
Powder + HNO ₃ (50%)	Light orange brown	Dark pinkish brown	Dark orange brown	Coffee brown
Powder + H ₂ SO ₄ (50%)	Light brown	Dark greenish brown	Dark brown	Dark greenish brown
Extracts:				
a) Petroleum ether (60-80°C)	Green	Bright orange	Greenish brown	Dark orange
b) Chloroform	Greenish brown	Bright orange	Dark brown	Orange brown
c) Ethyl alcohol (95%)	Orange brown	Mustard yellow	Dark brown	Orange brown
d) Water	Dark brown	Orange brown	Orange	Yellow brown

Table 2. Physicochemical characters of the stem of Vernonia elaeagnifolia DC. and market Chounggyah

Particulars	<u>Vernonia elaeagnifolia</u> DC. (%)	<u>Market Chounggyah</u> (%)
Loss of weight on drying	12.6	9.4
Total ash	6.6	3.3
Acid-insoluble ash	0.5	0.6
Water-soluble ash	5.2	1.6
Extractive values		
a) Petroleum ether (60-80°C)	4.00	0.33
b) Chloroform	0.46	0.33
c) Alcohol	2.50	0.83
d) Water	3.30	1.75

proteins, resins, saponins, starch, steroids, tannins and amino acid according to standard phytochemical

methods and the findings are recorded in Table 3.

THIN LAYER CHROMATOGRAPHY

Rf values of chloroform and alcoholic extracts of both the stem powders were studied under ultra-violet light according to standard chromatographic methods and the results are presented in Table 4.

DISCUSSION

Chounggyah, cultivated in Traditional Medicine Hospital, Mandalay, herbal garden is a climbing perennial plant, with long finely striated branches; alternate linear-oblong or elliptic-lanceolate leaves. Hoary inflorescence consisting of 3- to 5-flowered heads in narrow, short loose panicles and fruit with white persistent papus.

Table 3. Preliminary phytochemical screening of the extracts of the stem of Vernonia elaeagnifolia DC. and market Chounggyah

Extract	Fixed oils	Carbohydrate	Glycoside	Phenols	Protein	Resin	Saponin	Starch	Steroid	Tanin	Amino acid
Petroleum ether extract											
a) <u>Vernonia elaeagnifolia</u> DC.	+	-	-	-	-	-	-	-	+	-	-
b) Market Chounggyah	-	-	-	-	-	-	-	-	+	-	-
Chloroform extract											
a) <u>Vernonia elaeagnifolia</u> DC.	-	+	-	-	-	-	-	-	-	-	-
b) Market Chounggyah	-	+	-	-	-	-	-	-	-	-	-
Alcohol extract											
a) <u>Vernonia elaeagnifolia</u> DC.	-	+	+	+	-	+	-	-	-	+	+
b) Market Chounggyah	-	+	-	+	-	+	-	-	-	+	+
Water extract											
a) <u>Vernonia elaeagnifolia</u> DC.	-	+	+	+	-	-	+	-	-	+	+
b) Market Chounggyah	-	+	-	-	+	-	+	+	-	-	+

M.B. All extracts were negative for resinified volatile oils, flavonoids and alkaloids

Table 4. Thin layer chromatography of the stem of Vernonia elaeagnifolia DC. and Market Chounggyah

Extract	Solvent System	Observation under long-wave ultra-violet light			
		Vernonia elaeagnifoloid DC.		Market Chounggyah	
		No. of spot(s)	Rf value(s)/Colour	No. of spot(s)	Rf value(s)/Colour
Chloroform	Chloroform:Methanol 95:1	-	-	5	0.67 (Bb)
				5	0.42 (C)
	Chloroform:Ethyl acetate 1:1				0.29 (n)
					0.25 (n)
					0.18 (n)
Ethanol	Chloroform:Ethyl acetate 1:1	1	0.94 (O)	4	0.93 (Lb)
					0.73 (Bb)
	Benzene:Ethyl acetate 95:1	3	0.65 (Lr)	3	0.49 (Lb)
			0.51 (Lb)		0.10 (Bb)
			0.37 (Lr)		0.03 (W)
	Chloroform:Methanol 9:1	-	-	4	0.84 (Wb)
					0.55 (Pb)
					0.43 (Lg)
	Benzene:Ethyl acetate 95:1	-	-	3	0.25 (Yb)
					0.64 (Lp)
				0.15 (Lb)	
			0.04 (Bb)		
N.B. - Bb = Bright blue Lb = Light blue Lr = Light red W = White					
Bc = Bright cream Lg = Light grey O = Orange Wb = Whitish blue					
C = Cream Lp = Light purple Pb = Purplish blue Yb = Yellowish blue					

These characters are in agreement as mentioned in Hooker, 1887 [2]. From the morphological characters seen in Figure 1, this plant was identified as Vernonia elaeaginifolia DC. From the comparative macroscopic features and microscopic characters (Fig. 2 and Fig. 3), fluorescence characteristic presented (Table 1), physicochemical characters tabulated (Table 2), phytochemical data (Table 3) and the Rf values, number of spots and colour characters of the two extracts (Table 4) it was observed that market Chounggyah is entirely different from Vernonia elaeaginifolia DC.

Therefore, researchers should be aware of medicinal plant drugs with the same Myanmar name used for different plants making it difficult when collecting the correct authentic samples.

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Widal agglutination test reaction in patients with falciparum malaria

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Serum samples from 115 patients with falciparum malaria who attended Tharyarwady Civil Hospital during summer and rainy seasons of 1991 were studied. Of these, 71 patients were followed at Day (7) of admission to the hospital. The sera were tested by tube agglutination for antibodies against O and H suspension of enteric fever organisms (Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B). 3 samples of sera collected from patients with parasite density (1+) and suffering from uncomplicated malaria showed agglutination titre +1/40 to O and H antigens of Salmonella typhi. One serum sample collected from a patient with parasite density (2+) and suffering from uncomplicated malaria showed agglutination titre +1/40 to O antigen of Salmonella typhi. All the remaining samples showed -1/40 agglutination titre to Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B, O and H antigens. There was also no increase in agglutination titres at Day (7) samples. Thus there may be no relation between Widal agglutination test reaction and clinical severity or parasite load of falciparum malaria patients.

INTRODUCTION

Severe and life threatening infection may arise in patients with severe falciparum malaria. Gram negative septicaemia may occur without any evidence of focus and with no obvious relationship with malaria infection. Out of 175 patients with strictly defined cerebral malaria, Gram negative bacteria had been cultured in 10 patients (Escherichia coli in three, Pseudomonas aeruginosa in one and uncharacterized bacteria in six) [1].

Association with E. coli and Clostridium perfringens septicaemia was also reported [2], a patient with Pseudomonas aeruginosa septicaemia were also described from Denmark [3].

In Gambia, Mabey et al. [4] studied the association between malaria and Salmonella septicaemia. They found that young children with non-typhoid Salmonella septicaemia were more anaemic and more likely to have evidence of recent malaria than children of the same age with other form of septicaemia.

In a prospective hospital based study from Myanmar [5], endotoxin was detected in the blood of 18 out of 20 patients with complicated falciparum malaria and in all five patients with uncomplicated malaria but not in any out of five healthy volunteers. There were four deaths among the 18 patients with complicated malaria and endotoxaemia. The association between malaria and bacterial infec-

tion was reviewed by WHO 1990 [6]. In Myanmar many physicians have the view that malaria patients may have concomittant typhoid infection. Thus this study, Widal agglutination test reaction in patients with falciparum malaria is needed.

PATIENTS AND METHODS

The study was done at two different seasons.

(A) Summer season (Jan to Feb 1991)

Widal agglutination test reaction was studied on forty nine patients with falciparum malaria who attended the Out-patient Department of Clinical Research Unit (Cerebral and Complicated Malaria) at Tharyarwady Civil Hospital. They were sub-grouped in relation to parasite density (+ to ++++ of Bruce Chwatt scale) and clinical severity (uncomplicated, complicated, and cerebral) according to WHO classification.

Of these patients, thirty were also followed at Day Seven of admission to hospital. Serum samples collected from the patients were stored at (-10°C) and tested at National Health Laboratory for Widal agglutination test reactions.

The patients' serum were tested by tube agglutination for antibodies against H, O suspension of enteric fever organisms S. typhi, S. paratyphi A and S. paratyphi B. As the results from summer showed no correlation between Widal reaction and falciparum malaria, the study was repeated at the rainy season when enteric infection is common in Myanmar.

(B) Rainy season (June to July 1991)

Sixty-six serum samples were collected from patients with falciparum malaria. Of these, 41 samples were collected again at Day 7 of admission to hospital.

The serum samples were stored and tested by Widal agglutination test as above.

RESULTS

Three samples of sera collected from patients with parasite density (1+) and suffering from uncomplicated malaria showed agglutination titre +1/40 to O and H antigens of Salmonella typhi. One serum sample collected from patient with parasite density (2+) and suffering from uncomplicated malaria showed agglutination titre +1/40 to O antigen of Salmonella typhi. All the remaining samples showed -1/40 agglutination to Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B, O and H antigens. There was also no increase in agglutination titres at Day 7 samples.

DISCUSSION

During the summer study, 3 out of 49 patients showed low agglutination antibody to somatic and flagella antigen of typhoid bacilli. In none of the patients there was increase in antibody level in day (7). The study was repeated at the rainy season when the enteric infection is common in Myanmar. During the second study, there was also only one patient with low agglutination antibody level to somatic antigen of typhoid bacilli. In none of the patients there was an increase in antibody level at day (7).

Such low level of agglutination reaction is common among Myanmar living in general community [7].

Thus we wish to conclude that there is no association between reactions to Widal agglutination test and parasite load or clinical severity of P. falciparum malaria up to day 7 of admission to the hospital

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SHORT REPORT

**Determination of malaria parasite rates in Anophelines
(Diptera : Culicidae) by ELISA from two areas of Myanmar**

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Malaria is widely distributed in various hilly, coastal and plain regions of Myanmar and has been a national priority health problem since 1978 [1]. Malaria transmission is ultimately dependent upon the sporozoite rate: the proportion of female anopheline mosquitoes with sporozoites in their salivary glands. The entomological inoculation rate, defined as the product of the sporozoite rate and the man-biting rate is an important parameter for quantifying malaria transmission. Assessment of this parameter will become important as sites are investigated for malaria vaccine trials [2].

Previously, the immunoradiometric assay (IRMA) was developed using monoclonal antibodies against malaria species. The ELISA was later adapted for the same purpose and proved equally reliable. We have pursued establishing an ELISA sporozoite detection system since such a system has not been used before in determining *Plasmodium* infection rates for anophelines of Myanmar. It does not require radioactive reagents or a gamma counter for analysis. Moreover, sporozoites can be identified by species, specimens can be held in storage (dry or frozen) before processing, the reagents used are stable for long periods of time and tests in 96-well microtitre plates can be read either visually or quantified with a spectrophotometer. In addition to not having these advantages, dissec-

tions techniques require careful microscopic examination of freshly-caught anophelines by trained personnel, usually under difficult field conditions.

The objective of this short paper is to report the introduction of an ELISA sporozoite system in our Department using ELISA test kits provided by WHO/TDR for the An. dirus project ID:890372 and the important vector anophelines (*An. dirus*, *An. minimus* and *An. annularis*) collected from two areas of Myanmar.

The first area was a hyperendemic malarious area (Tha-bye-wa village) in Oktwin township, Pegu Division (approx. 270 km north of Yangon) with a spleen rate of 68.9% and an average enlarged spleen (AES) of 1.87 (in age group 2-9 years). The second area was a mesoendemic malarious area (Sedawgyi village), Madaya township, Mandalay Division (approx. 640 km north of Yangon) with a spleen rate of 37.8% and an average enlarged spleen of 1.84.

The identified anophelines were killed immediately or after oviposition depending on the need for isofemale lines. The head-thorax portions of the mosquitoes were cut from the abdomen and dried according to the standard method [3]. In our laboratory, these specimens were stored at -20°C before carrying out ELISA tests 2-4 months later. The steps in the sandwich ELISA for detection of sporozoites were as mentioned in the

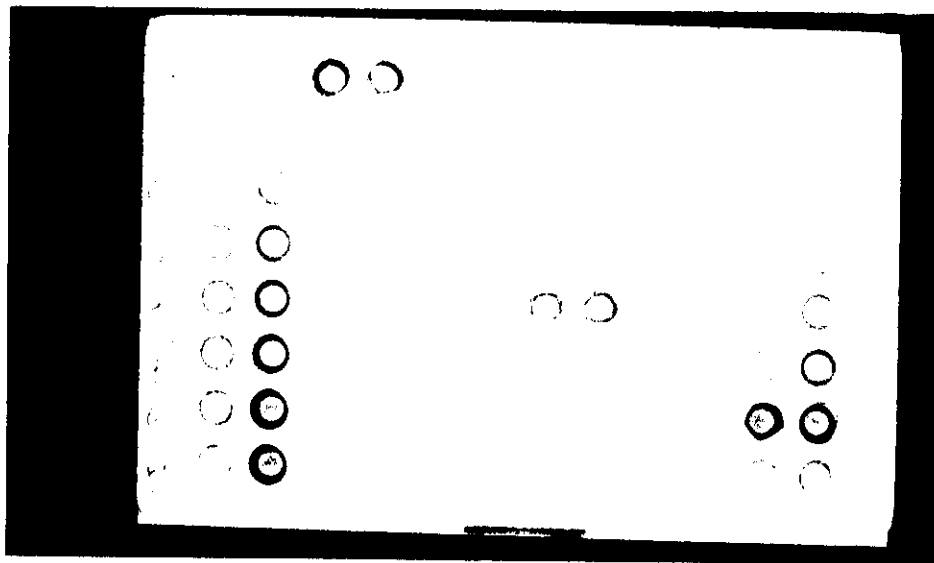


Plate 1. ELISA plate showing 3 pairs of wells positive for Plasmodium falciparum circumsporozoite protein

manual provided in the ELISA test kits.

Anophelines from these two areas were compared and the results indicated that 3.7% (4 of 106) of An. dirus from Oktwin township and 6.3% (5 of 79) An. minimus and 2.2% (2 of 90) An. annularis from Madaya township were positive for P. falciparum and P. vivax. The positive specimens were confirmed as positives. This is the first time that ELISA assays were used in determining Plasmodium infection rates for anophelines of Myanmar.

Similarly, Plasmodium sporozoite ELISA tests on head-thorax samples of 79 An. minimus and 90 An. annularis from Sedawgyi village, Madaya township, Mandalay Division showed that 6.3% (4 of 79) and 2.2% (2 of 90) were positive during the initial screening. One An. minimus and one An. annularis were positive for Plasmodium falciparum circumsporozoite protein and three An. minimus and one An. annularis were

positive for Plasmodium vivax circumsporozoite protein. All positive specimens were tested again and all were confirmed as positives.

An. dirus and An. minimus are the primary vectors in Myanmar. An. annularis, a secondary vector was also collected from Sedawgyi village, Madaya township and the sporozoite rate detected was 2.2% (2 of 90). Sporozoite rate for An. annularis was officially reported in Myanmar only prior to 1930 [4]. This is the first time since 1930 that sporozoites were detected in An. annularis and officially reported (although from dissection records of 1976, 1 An. annularis (our of 21) was found positive for sporozoites in Akyab (Marcus, personal communication). Although this vector is zoophilic, it will feed on man in the absence of cattle. This vector had been detected in the Yangon area (Sebastian, personal communication) and it is likely that these mosquitoes can become infected from imported

gametocytes carriers. This needs further studies.

In our present study, both the sensitivity and specificity of the ELISA assays were tested and were found to be good. Contrary to the findings on studies carried out in the Egyptian Oases [5], our confirmatory tests revealed that all the positive Plasmodium species were positive again for the same species and 3 known sporozoites positive An. dirus were shown to be positive in addition to the standard positive controls showing positive results. All the sporozoites negative An. dirus mosquitoes and the standard negative controls showed up as negatives in the ELISA assays.

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SHORT REPORT

Malaria in Kyaingtone

A six month study on hospital admitted patients

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In Myanmar, malaria is a focal endemic disease. It is neither uniformly prevalent nor equally distributed. It is mainly a rural problem.

The clinical signs and symptoms of malaria varies with the different areas of study. Aung Than & Ye Naing [1] had reported a high number of retinal haemorrhage among patients with falciparum malaria from Military Hospital. But such clinical syndrome was not often observed among the civilian patients from Tharyarwady [2].

Hence the clinical presentations of malaria in Kyaingtone (Eastern Shan States) which is situated at 2700 feet above sea level and 740 miles away from Yangon was studied.

The hospital records of the whole year of 1987 was studied.

The period when there is high prevalence of malaria (1st January 1988 to 30th June 1988) was chosen for clinical study. All the patients who attended the out-patient clinic, medical unit and paediatric units of the hospital were included in the study. A preformed data collection charts were used for the data analysis of in-patients.

According to 1987 hospital statistics the proportion of clinically suspected malaria among out-patients was 24.64%. Among the in-patients the

proportion of clinically suspected malaria was 52.62%. The highest number of patients was recorded at January.

During 1988, (6 months malaria season), 1303 patients were admitted to medical unit and 645 patients to Paediatric Unit. The proportion of adult and children was 2:1. Among the adult patients the proportion of male and female was 10:9. The spleen rate among the adult patients was 35.4% and in children (2-9 years) was 50.7%.

Slide positivity rate among the in-patients of clinically suspected malaria was 20.10% in adults and 30.12% in children. Due to various reasons species was not identified.

Among the adults with severe and complicated malaria the prevalence between male and female was 10:9. The prevalence of jaundice was more common in males (18.9%) when compared to females (7.6%). Shock syndrome was present in only one female patient.

Among adults, only 17 (1.7%) of patients were associated with cerebral malaria. Of these, 9 patients (52.9%) expired of the disease. In children 13 (2.0%) patients have cerebral malaria. Of these one child (7.7%) expired of the disease. In both adults and children, cerebral malaria

was not associated with anaemia. Cerebral malaria was associated with splenomegaly in children ($p < 0.05$).

There were eleven deaths among adults (6 males and 5 females) and five deaths in children. The case fatality rate among patients with complicated malaria was 6.8% in adults and 4.3% in children.

Among the adults, death was significantly associated with cerebral malaria ($p < 0.01$). There was no association between anaemia, jaundice, bleeding episode, splenomegaly and death.

Among the children, death was not associated with cerebral malaria or splenomegaly. It was significantly associated with severe anaemia ($p < 0.01$) jaundice ($p < 0.01$) and bleeding episodes ($p < 0.02 > 0.01$).

Kyaingtone is situated at Shan Plateau. During winter especially during December to January temperature is very cool and may fall to the level as -0°C . This area is full of hills and valleys. But most of the forest at the hills had been cut down or burnt down for plantation. During 1987, the incidence of clinically suspected malaria (CSM) among out-patients of Kyaingtone Hospital was 24.6% and among the in-patients was 52.6%. In same year the prevalence of CSM cases at all hospitals of Myanmar was 5.3% among the out-patients and 16.9% among the in-patients. At the Shan States in the same year, the prevalence of CSM among the out-patients was 13.4% and among in-patients was 27.4% [3]. Therefore the prevalence of malaria was high in Kyaingtone when compared to the whole country or to Shan States.

In this study, highest prevalence of malaria was recorded between January and June summer season. This is

different from the report of Defence Service General Hospital (DSGH), Yangon [4], where the prevalence of malaria was highest in June. It is also different from the data of Tharyarwady where the highest number of admissions was in May and June [2], Kayin State - March, April and May [5] and Mandalay - July, August and September [6]. This may be due to the differences in climatic condition and status of travelling among the residents of the area.

In this study, the proportion of children admitted to hospital was nearly 50% and among adults the male and female proportion was 10:9. Such high proportion of children and female was not observed at Tharyarwady [2] or in Kayin State [5] or Mandalay [6]. This difference may be due to local transmission in the town and at the periphery.

In this study only 1.3% of adults and 2% of the children had cerebral malaria. Such low prevalence of cerebral malaria was not observed in DSGH [4], Tharyarwady [2], Kayin State [5] or Mandalay [6]. This may be due to development of partial resistance to the disease by most of the population of the township.

Hence malaria in Kyaingtone is unique. The epidemiology of the disease and vectors of transmission are needed to be studied.

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**Concentration method of detecting Plasmodium falciparum using
microcentrifuge and heparinised capillary tubes**

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Sixty malaria patients were studied. Thick blood films were prepared from the finger tips of the patients as usual to detect malaria parasites in blood. A sample of blood is also collected from the same finger tip with heparinised capillary tubes at the same time. The tubes were centrifuged at 10,000 rpm for 5 minutes. The capillary tube was then broken into 3 pieces. One at upper part of the red cell column just below the white cells and another at the base of the red cell column. Blood collected from these two samples were stained together with samples from finger tips using usual standard procedure for the detection of malaria parasites. Blood collected from the uppermost part of red cell column yield the highest parasite density. The use of capillary tube is not an extraburden to the work load of the malaria unit and special instruments are also not necessary. This method may help in the detection of patients with low level of parasites in peripheral blood.

INTRODUCTION

Malaria slides are usually prepared from the blood collected from the finger tips of the patients. Often there is no co-relation between the severity of the disease and parasite count. At times there are malaria patients with no detectable parasites in peripheral blood at time of admission to hospital. The diagnosis is at times confirmed only from post mortem studies. Hence concentration method of detecting P. falciparum parasite is needed to help in diagnosis of patients.

Red cells containing plasmodium parasites may be less dense than the normal ones. In capillary tubes after centrifugation the parasitised cells may be collected just below the leucocytes at the top of the

erythrocyte column. Hence heparinised capillary tubes may be useful to concentrate the parasites in the red cells.

PATIENTS AND METHODS

Sixty patients who attended the Tharyarwady Civil Hospital during 1990-91 malaria seasons were chosen for study.

Thick blood films were prepared from the finger tips of the patients as usual to detect malaria parasites in blood. A sample of blood was also collected from the same finger tips with heparinised capillary tubes at the same time. Heparinised capillary tubes from Howksley and Sons Ltd. England were used for the test. The tubes were centrifuged at 10,000

rpm for 5 minutes, the pack cell volume of the patients were recorded for clinical use.

The capillary tubes were then broken into 3 pieces one at upper part of the column just below the white cells and another at the base of the red cell column. Blood collected from these methods were stained with usual standard procedure for detection of malaria parasites. The number of parasites in the 3 samples of blood were graded according to Bruce-Chwatte Scale.

RESULTS

The blood collected from the upper part of RBC column just below the white buffy coat, showed highest concentration of parasites in all 60 samples. Samples collected from finger tips is always second and samples collected from base of RBC column is always third, according to Bruce-Chwatt Scale. The mean level of parasite, according to Bruce-Chwatt Scale in the 3 groups of samples were 1.58 ± 0.75 , 0.91 ± 0.69 and 0.35 ± 0.57 respectively. The differences between 3 groups is highly significant ($p < 0.01$). Three samples which showed negative by routine finger tip method showed one plus in samples collected from upper part of RBC column of capillary tube.

DISCUSSION

In this study, the samples of blood collected from the upper part of the capillary tube of RBC column just below white cell buffy coat had the highest yield of parasites. Blood sample collected from finger tips were second higher, while the RBC collected from the base of the column had comparatively low level of para-

sites. This confirm our hypothesis that red cells containing plasmodium parasites are less dense than the normal ones and concentrate just below the leucocytes at the top of the erythrocyte column.

Using the conventional thick film method to detect a small number of parasites requires a highly skilled trained microscopist who must looked up to 100 fields in each slide. To diagnose a negative film, the examination time may take up to 10 minutes per slide. With this method the positivity rate is much increased. Using a modified haematological apparatus (the QBC tube) and similar procedure using centrifuged blood, Spielman [2,3] had also reported the centrifuged blood as eight times more sensitive than conventional microscopy.

Thus the use of capillary tube may help in the detection of patients with lower level of parasites in peripheral blood. Moreover, the use of the capillary tube is not an extra burden to the laboratory who is looking after the malaria patients. PCV is essential to detect the degree of anaemia on all patients with falciparum malaria. Our unit has been measuring PCV daily on all malaria patients while they are staying at the hospital [4].

Further study is needed using thin blood smears for identification of malaria species.

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We wish to thank Township Medical Officer (1) and staff of Tharyarwady Civil Hospital for all their help.

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