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Serum cortisol response in falciparum malaria

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Thirty patients with uncomplicated malaria, thirty with cerebral malaria and thirty controls were included in the study. The serum cortisol level of patients was determined at Day 0, Day 3 and Day 7 by using radioimmunoassay method. The mean serum cortisol level of patients with uncomplicated and cerebral malaria were 532.2 ± 120.9 n mol/L and 521.5 ± 80.5 n mol/L respectively. Among controls, the mean level was 398.8 ± 141 n mol/L. There was a significant rise of serum cortisol levels in patients with falciparum malaria when compared to controls at Day 0 (day of admission). There was no significant difference between uncomplicated malaria and cerebral malaria. There was also no significant difference between different days of treatment. It was found that no cortisol insufficiency in falciparum malaria during acute and convalescent stages of illness. Relationship of serum cortisol level with parasite density and patient outcome was also studied and both of these variables bear no relationship with serum cortisol level.

INTRODUCTION

Corticosteroids were once thought to reduce the cerebral edema believed to be associated with cerebral malaria. From 1967 to 1982, they were the most commonly administered adjunctive therapy for cerebral malaria [1-3]. But clinical studies failed to confirm the value of using corticosteroids [4-6]. Serum cortisol level of patients with some infectious disease can have low serum cortisol level, for example, cortisol insufficiency in Korean hemorrhagic fever cases with or without pituitary atrophy [7].

Moreover, direct measurement of serum cortisol level in falciparum malaria has never been reported in literature except a study by Tin Shwe *et al.* (1996) who studied serum cortisol level in patients with uncomplicated and cerebral malaria [8]. But their study was limited to 10 patients in each group. Therefore, serum cortisol level of falciparum malaria cases should be estimated in larger sample size and it might be helpful for better understanding of the

role of corticosteroids in management of cerebral malaria. In the present study, relationship of serum cortisol level with parasite density and patient outcome were also investigated.

MATERIALS AND METHODS

Study design

This study was prospective and longitudinal one. Two groups of patients and one group of normal persons for controls were chosen for the study. The first group consisted of 30 patients with uncomplicated malaria and the second group 30 patients with cerebral malaria admitted to medical ward of NOGH during May 1996 to April 1997. Control group involved 30 apparently healthy volunteers who were the attendants of patients.

Methods

Patients of both sexes with asexual forms of *P. falciparum* identified on blood film were

included in the study. Cerebral malaria was defined as the patient with unarousable coma not attributable to other cause in a patient with falciparum malaria. Coma should persist for at least 30 minutes after a generalized convulsion for the diagnosis of cerebral malaria. Those patients with no features of complicated malaria (WHO, 1990) such as jaundice, acute renal failure, anemia etc. were taken as uncomplicated malaria patients. Nature of the study was explained to the patients and informed consent was obtained.

The sera of the patients were collected on Day 0, Day 3, and Day 7. Thirty sera samples of healthy volunteers were also collected as controls. We determined the serum cortisol level using commercially available RIA Kits from Diagnostic Products Corporation (DPC), Los Angeles, CA, USA. It is a competitive radio-immunoassay in which ^{125}I -labeled cortisol competes with cortisol in the patient sample for antibody sites. The lower detection limit is 8.28 nmol/L and both intra and inter-assay precisions are less than 10 percent. We performed all analyses in duplicates and Packed Auto-Gamma Scintillation Spectro-meter, Model 5230 was used for radioactive measurements.

Statistical analysis

Data analysis was performed with SPSS Ver 3.0 software on an IBM PC computer. Data were expressed as mean $\pm$ (SD). Comparisons of serum cortisol level between groups and different days of treatment were made using Student's 't' test. Differences were considered significant if $p < 0.05$.

RESULTS

Out of 60 patients 30 with uncomplicated falciparum malaria and 30 with cerebral malaria), 44 (73.3%) were males and remaining 16 (26.6%) were females. Minimum age of patients in this study was

14 years and maximum 74 years. Average duration of stay in hospital for uncomplicated and cerebral malaria patients were 7 days and 10 days respectively.

The mean serum cortisol level of patients with uncomplicated malaria at Day 0 (day of admission) was 532.2 ± 120.9 nmol/L. The mean cortisol level of patients with cerebral malaria was 521.5 ± 80.5 nmol/L and among controls, the mean level was 398.8 ± 141 nmol/L. There was significant rise of cortisol level in both groups of patients with malaria when compared to controls at the day of admission. There was no significant difference between uncomplicated malaria patients and those with cerebral malaria (Table 1).

Table 1. Serum cortisol level in falciparum malaria cases and controls

Group	No. of patients	Serum cortisol (mean $\pm$ SD)	P-value
Control	30	398.8 ± 141 nmol/L	
Uncomplicated	30	532.2 ± 120.9 nmol/L	*0.0001
Cerebral	30	521.5 ± 80.5 nmol/L	**0.0002

*Control Vs uncomplicated

**Control Vs cerebral

There was also no significant difference between different days of illness in the two groups of malaria patients (Table 2).

In uncomplicated malaria, mean serum cortisol level of patients with parasite count $< 10,000/\mu\text{l}$ was compared with those of parasite count $> 10,000/\mu\text{l}$. There was no significant difference between these two groups.

The same comparison was also done in cerebral malaria cases and found no significant difference (Table 3). We also compared serum cortisol level of fatal and non-fatal cases of cerebral malaria, and found that difference was not statistically significant (Table 4).

Table 2. Serum cortisol level in falciparum malaria cases between different days of illness

Group	Serum cortisol (mean $\pm$ SD) in nmol/L			p-value
	Day 0	Day 3	Day 7	
Uncomplicated	532.2 $\pm$ 120.9	486.9 $\pm$ 106.2	490.2 $\pm$ 52.5	0.14
Cerebral	521.5 $\pm$ 80.5	490.0 $\pm$ 120.4	499.0 $\pm$ 130.4	0.54

Table 3. Relationship between parasite density and serum cortisol level in uncomplicated and cerebral malaria

Parasite count/ l	No. of patients	Serum cortisol (mean $\pm$ SD) nmol/L	p-value
<10,000	21	528.2 $\pm$ 120 nmol/L	*0.523
>10,000	9	498.0 $\pm$ 115.8 nmol/L	
<10,000	11	520.9 $\pm$ 105.5 nmol/L	**0.764
>10,000	19	505.6 $\pm$ 147.7 nmol/L	

*Uncomplicated

**Cerebral

Table 4. Comparison of serum cortisol level in non-fatal and fatal cases of cerebral malaria

Group	No. of patients	Serum cortisol mean $\pm$ SD	p-value
Non-fatal	25	516.0 $\pm$ 80.5	0.643
Fatal	5	534.9 $\pm$ 97.3	

DISCUSSION

In our study, there was significant rise of serum cortisol level in both uncomplicated and cerebral malaria cases as compared to controls on admission. Moreover, there was no patient with cortisol level less than mean level of controls at Day 0, Day 3 and Day 7. Serum cortisol level between different days of illness also showed no significant difference.

Our findings were in agreement with the study by Tin Shwe *et al* (1996) who reported that there was no cortisol insufficiency in falciparum malaria cases during acute and convalescent stages of illness [8]. But this is in contrast with the study by Lim *et al* (1986) who showed that serum cortisol level of patients with some infectious disease can have low serum cortisol level, for example cortisol insufficiency in Korean hemorrhagic fever cases with or without pituitary atrophy [7].

During an illness, a close relationship between the release of cortisol and disease severity had been documented [9]. The present study demonstrates higher serum cortisol levels during illness as compared to controls. Although increases up to 5-fold have been reported in patients with pneumonia, bacterial meningitis and PUO cases [10], we observe only two fold increase in cases of falciparum malaria. This is in agreement with the study by Inamo *et al*, where a two-fold increase in cortisol levels during febrile illness was reported [11].

Clinical trials using both low and high dose dexamethasone showed no benefit in treatment of cerebral malaria. Moreover steroid treatment could lead to an increased incidence of steroid related side effects [12]. Evidence from clinical studies in man also did not support the hypothesis that cerebral malaria resulted from an inflammatory breakdown in permeability of blood brain barrier resulting in cerebral edema [13]. Thus Wyler in 1988 reversed the literature and concluded that corticosteroids should not be used in cerebral malaria as this treatment now lacks theoretical basis and clinical evidence of efficacy and cause a risk of side-effects [14].

Therefore, all these previous findings also support our present finding that it is not necessary to supplement corticosteroids for the treatment of cerebral malaria.

Our study revealed no significant relationship between parasite density and serum cortisol level in both uncomplicated and cerebral malaria cases. Thus, we may conclude that parasite density bears no relationship with serum cortisol level in falciparum malaria.

No significant difference was found in serum cortisol level between uncomplicated and cerebral malaria cases. Comparison between fatal and non-fatal cerebral malaria also showed no significant difference. These findings suggested that serum cortisol level does not have any relationship with clinical severity and patient outcome.

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Bacterial species isolated from fish/prawn sauce (Ngan-pya-yei) and prepared fish paste (Ngapi-yei-gyou-phyaw) from street vendors in Yangon area

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From early August to the end of November 1998, a random sampling of 46 samples of ngan-pya-yei, 37 samples of ngapi-yei-gyou-phyaw from small food shops from Dagon, Latha, Papedan, Kamayut, Thamaing, Mayangone, Mingaladon, North Okkalapa and Htaukkyant townships and 22 raw samples of ngapi-yei-gyou from the nearest bazaars of respective townships were tested for bacteriological analysis. Coliforms, faecal coliform and *Vibrio* species were isolated from 8.7%, 2.17% and 30.44% respectively from ngan-pya-yei specimens. Coliforms, faecal coliforms and *Vibrio* species were isolated from 91.89, 62.16 and 29.73% respectively from ngapi-yei-gyou-phyaw specimens. Moreover, from raw ngapi-yei-gyou, the isolation rate of coliforms, faecal coliforms and *Vibrio* species were found to be 81.82%, 50% and 22.73% respectively. The isolated bacteria were mostly *Escherichia coli*, *Klebsiella aerogenes* and *Proteus vulgaris* of Enterobacteriaceae. Isolated *Vibrio* species, differentiated by salt tolerance, oxidase and some biochemical tests, were identified as *V. vulnificus*, *V. mimicus*, *V. fluvialis* and *V. alginolyticus*. Thus, these side dishes, which were easily available and fond by many persons, may serve as a reservoir and a vehicle in transmission of enteric infections.

INTRODUCTION

One of the major health problems in the large number of gastrointestinal cases and outbreaks throughout the year contribution to high morbidity in many parts of the world. It is resulted by malnutrition; parasitic infestations and presumably only *Salmonella*, *Shigella*, EPEC and *Vibrio cholerae* are the potential bacterial pathogens. In Japan, during the last decade bacteria caused 64 to 86% of those outbreaks. According to the morbidity statistics from SEAMIC Health Statistics, 1998 [1], typhoid and paratyphoid fever, bacillary dysentery and bacterial food poisoning were important health problems in Indonesia, Malaysia, Philippines and Thailand and even Japan had faced these problems. The toxicity effect of contaminated food has been reported [2,3]. *Vibrios* were commonly found pathogens in

Vietnam [4], Indonesia [5] and Bangladesh [6]. In Myanmar diarrhoea and dysentery stood in the fourth position in National Health Plan (1996-2001) [7] and the role of bacteria in environment, especially in foods need to be explored accordingly. Therefore, this study was performed to determine the contamination of bacterial pathogens in ngan-pya-yei and ngapi-yei-gyou.

Objectives: To study the bacterial contamination of ngan-pya-yei and ngapi-yei-gyou-phyaw from street vendors.

Specific objectives:

1. To identify the presence of coliforms and faecal coliforms from ngan-pya-yei and prepared ngapi-yei-gyou-phyaw from street vendors.
2. To determine the contamination of bacteria in raw ngapi-yei-gyou.

MATERIALS AND METHODS

Sample collection

A descriptive study was done during August to November 1998 and the samples were collected from nine areas of Yangon (Dagon, Latha, Papedan, Kamayut, Thamaing, Mayangone, Mingaladon, North Okkalapa and Htaukyant). Small shops, which sell either mohinga and/or athoke, were chosen for collection of 46 samples of ngan-pya-yei; whereas, shops selling rice and curry were chosen to obtain 37 samples of prepared ngapi-yei-gyou. Twenty-two samples of raw ngapi-yei-gyou were collected from small bazaars of these townships. The samples were collected in new sterile plastic bags, used cold chain transportation and processed within 4-6 hours.

Determination of coliforms and faecal coliforms

It was processed according to the method of World Health Organization [8]. Multiple tube method using MacConkey broth (Purple) in double and single strength was done. It was then confirmed by Brilliant green bile broth. The enteric pathogens were isolated from these broths after culturing onto MacConkey agar and Salmonella-Shigella agar.

Determination of bacterial pathogens

It was determined by the method of WHO [9] using MacConkey Agar (MA), Nutrient Agar (NA), Salmonella-Shigella (SS) Agar, thiosulphate Citrate Bile Salt Sucrose Agar (TCBS). Selenite F broth and alkaline peptone water (6 hr) for secondary isolation. Confirmation of pathogens were done biochemically using Triple Sugar Iron Agar (TSI), Sulphide Indole Motility (SIM) Agar, Lysine Iron Agar (LIA) Christensen's Urease test and Oxidase test. Serology was also done whenever necessary.

RESULTS

Distribution of coliforms, faecal coliforms and vibrios

The distributions of coliforms, faecal coliforms and *Vibrio* species from ngan-pya-yei were found to be 10.87%, 2.17% and 30.44% respectively. For the prepared ngapi-yei-gyou, coliforms were isolated from 91.89%, faecal coliforms from 62.16% and *Vibrio* species from 29.73%. When raw samples of ngapi-yei-gyou were tested, 81.82% were contaminated with coliforms, 50% with faecal coliforms and 22.73% with *Vibrio* species (Fig. 1).

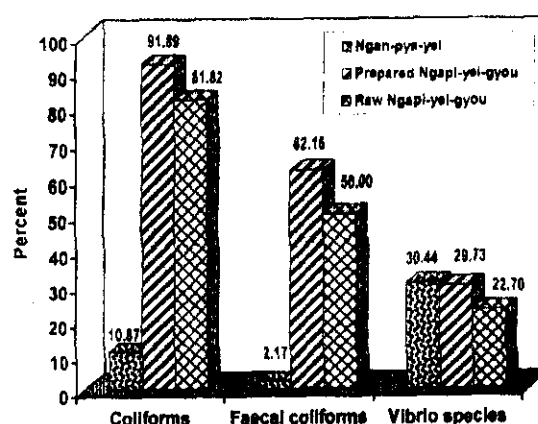


Fig.1. Distribution of coliform, faecal coliforms and *Vibrio* species

Comparative study upon the isolation of pathogens from different areas

Comparative study between the two areas, i.e. area - I (Dagon, Kamayut, Latha and Pabe-dan) and area - II (Thamaing, Mayangone, Mingaladon, North Okkalapa and Htaukkyant) is shown in Fig. 2. The coliforms and faecal coliforms were not isolated from ngan-pya-yei samples of area I, however, coliforms (15.63%) and faecal coliforms (3.13%) were isolated from samples of area II. The difference of isolation rate of *Vibrio* species was found to be not significant. It was also noted that the distributions of coliforms, faecal coliforms and *Vibrio* species in prepared and unprepared ngapi-yei-gyou from two tested

areas were found to be almost similar (Fig. 2).

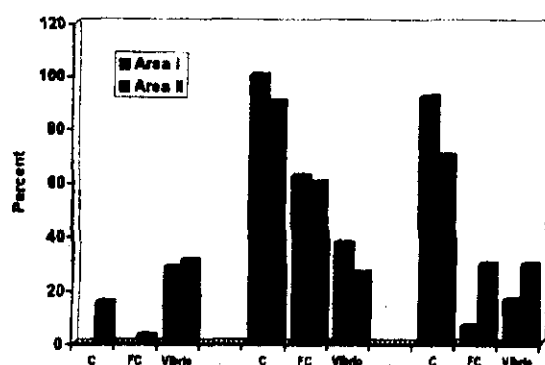


Fig.2. Comparative studies of the isolation of pathogen from different areas

Coliforms in the tested samples

The distribution of coliforms in the tested samples is shown in Table 1. The coliform counts of ngan-pya-yei were markedly lower than that of other two specimens, i.e. within the range of 2.2 to 5.1 MPN/100ml of sample. In prepared ngapi-yei-gyou, coliform counts of 2.2 MPN/100ml and 5.1 MPN/100ml were 2.7% each; 13.51% had the count of 9.2 MPN/100ml and 5.41% had the count of 16 MPN/100ml. It was noted that 67.57% had indeterminate count. In fresh ngapi-yei-gyou, the coliform counts varied from 2.2 to indeterminate, among them 68.18% had indeterminate coliform counts.

Table 1. Distribution of coliforms in tested samples

MPN/100ml or gm of the sample	Ngan-pya-yei	Prepared Nganpi-yei-gyou	Raw Ngapi-yei-gyou
2.2	4 (8.70)	1(2.70)	2(9.09)
5.1	1 (2.17)	1(2.70)	Nil
9.2	Nil	5(13.51)	1(4.55)
16	Nil	2(5.41)	Nil
Indeterminate	Nil	25(67.57)	15(68.18)

Figure in parentheses denote percentages

Distribution of faecal coliforms in the tested samples

Only 2.17% of ngan-pya-yei had faecal coliforms of 2.2 MPN/100ml. In prepared

ngapi-yei-gyou, 8.11% had the faecal coliform counts of 2.2 MPN/100ml; 10.81% had 5.1 MPN/100ml and 5.41% had 16 MPN/100ml respectively. In raw ngapi-yei-gyou, 4.55% had the count of 2.2 MPN/100ml; 9.09% with 5.1 MPN/100ml; and 18.18% with 9.2 MPN/ and 18.18% had indeterminate count (Table 2).

Table 2. Distribution of faecal coliforms in tested samples

MPN/100ml	Ngan-pya-yei	Prepared Ngapi-yei-gyou	Raw Ngapi-yei-gyou
2.2	1(2.17)	3(8.11)	1(4.55)
5.1	nil	4(10.81)	2(9.09)
9.2	nil	nil	4(18.18)
16	nil	2(5.41)	nil
Indeterminate	nil	14(37.84)	4(18.18)

Figures in parentheses denote percentages

Isolation of *Vibrio* species

All the *Vibrio* species differentiated by salt tolerance, oxidase and some biochemical tests were identified as *V. vulnificus*, *V. mimicus*, *V. fluvialis* and *V. alginolyticus*.

DISCUSSION

The grinding process introduces the surface contaminants into interior of the meat and may also warm the meat enough to encourage considerable bacterial multiplication. The interior of the meat is somewhat anaerobic, and fermentative organisms enriched for multiplication. In fish, the bacterial population will include many marine halophilic and psychrophilic forms. The "phosphorescence" of spoiling fish is due to the growth of luminescent marine bacteria (such as *Achromobacter*) on the surface. Shellfish gathered near a sewage outlet will contain numbers of sewage bacteria including both harmless and pathogenic enterobacteria. Vibrios from Canadian shellfish [10], oysters [11], raw fish meat [12], Indonesia [13] and influence of seafood handling [14] enlightened the importance of

bacterial contamination in food.

In our study, though the counts of coliforms and faecal coliforms of ngan-pya-yei were markedly lower than the prepared and raw ngapi-yei-gyou, the isolation rate of vibrios (30.44%) is not a good indicator for consuming *Vibrios* as it is well known that they could tolerate salt of various concentrations seems that these salted Myanmar traditional foods are suitable environment for them to harbour and plays an important role in transmission of *Vibrio* related gastro-enteritis.

In Myanmar the custom of eating tosaya and ngapi-yei-gyou is very common, especially in rural parts of the area. The water used for cleansing or (just dipping) may one of the main factor for the growth of contaminated pathogenic bacteria; especially if the water used is also contaminated. Though, the boiled ngapi-yei-gyou became free of any contamination, the ingredients used by consumers or the consumers themselves might play an important role for contamination. Thus, environmental hygiene always plays an important role in transmission of enteric infections.

From this study, it should be emphasis that hygiene behaviour and eating habits, such as repeated use of same dish after storage for several days, and sharing of same dish among many persons should be change to obtain the impact of health in our community.

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A prospective study to look for neuro-psychiatric adverse effects related to mefloquine given as prophylaxis or as a therapeutic agent in un-complicated malaria

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To confirm whether Mefloquine (MEPHAQUINTM – MEPHA) given either as a therapeutic agent in uncomplicated malaria, or as a prophylaxis is related with neuro-psychiatric adverse effects, a prospective study using a pre-tested carefully prepared neurological and psychiatric proforma was carried out in district Military Hospitals and Medical Battalions between May 1998 to April 2000. A total of 2243 cases were studied (1081 uncomplicated malaria patients on mefloquine therapy 1000-1500 mg stat dose, 249 subjects on mefloquine 250 mg plus sulphadoxine – pyrimethamine prophylaxis 1 tablet weekly alone for at least 6 months). The trained researchers who filled the questionnaire form of proformas carefully interviewed subjects. A detailed check list of neuro-psychiatric and non-neuro-psychiatric adverse effects were included. No severe neuro-psychiatric or non-neuro-psychiatric features were detected in both therapeutic as well as prophylactic groups and also in the control group. Mild transient symptoms included nausea, headache, dizziness and insomnia most of which subsided spontaneously within one week. Conclusion: Mefloquine (MEPHAQUINTM – MEPHA) was not associated with any severe adverse neuro-psychiatric effects in patients treated with therapeutic dosage of 1000-1500 mg stat or prophylactic dosage of 250 mg weekly for 6 months.

INTRODUCTION

Mefloquine hydrochloride was developed by the Walter Reed Army Institute of Research in collaboration with the UNDP/World Bank/WHO Special Programmed for Research and Training in Tropical Diseases (TDR) and the pharmaceutical company F. Hoffman-La Roche (ROCHE). Extensive clinical and field trials demonstrated mefloquine to be effective and safe for the prophylaxis and therapy of malaria when it was first

introduced more than 15 years ago (1983) [1]. It remains one of the most effective anti-malarial drugs against multi-drug resistant falciparum malaria, although resistance to it has now been reported in several areas especially in Thai-Cambodia and Thai-Myanmar border areas.

Given at an adult dose of 1500 mg, mefloquine (LARIAMTM – Hoffman-La Roche) was reported to be safe, symptoms commonly associated with its use, such as nausea, vomiting and dizziness were mild and transient [2-4].

Between 1985 and 1995, a total of 1574 neuro-psychiatric adverse events have been reported with the use of 1500 mg mefloquine (LARIAMTM) to the pharmaceutical company and WHO [5-7]. These included affective disorders, anxiety disorders, hallucinations, sleep disturbances and in a few people overt psychosis, toxic encephalopathy, convulsions and acute brain syndrome. Risks were more in Caucasians and Africans than Asians. Risk was highest in people with a neurological or psychiatric history.

The prevalence of "serious" neuro-psychiatric reactions is reported to be relatively low – 1:10,000 following prophylactic use and usually occurring early in the use of the drug.

Neuro-psychiatric symptoms were more with mefloquine given for treatment; the frequency is more – 1:200 to 1:1200 and also depending on ethnic origins and dose related.

The neuropsychiatry symptoms were reported to occur within 3 days in 73% of patients. 9% reporting onset 10 days or more after treatment.

The majority 78% reported resolution of symptoms within 3 weeks.

In fifteen years of experience with mefloquine (MEPHAQUINTM - MEPHA) in Myanmar, at the Clinical Research Unit (Malaria), Defense Services General Hospital (DSGH), no serious neurological features were noticed apart from some cases of transient dizziness, nausea, vomiting and diarrhoea.

The present study intends to interview the patients who have taken mefloquine (MEPHAQUINTM) for therapy or for prophylaxis and made detailed interviews inquiring specifically into any adverse effects.

Objectives

1. To find out the percentage of adverse symptoms (neuro-psychiatric and non-neuropsychiatric) following MephaquinTM therapeutic and prophylactic doses.
2. To find out the timing and duration of these symptoms.
3. To compare the results with those of subjects who are on the usual sulphadoxine-pyrimethamine weekly prophylaxis.

Site of study

1. Clinical Research Unit (Malaria), Defense Services General Hospital, Mingaladon.
2. No. 1 Military Hospital (300-Bedded), Myitkyina
3. No. 2 Military Hospital (300-Bedded), Sittwe
4. No. 11 Military Hospital (100-Bedded), Mongsat

Study period

May 1998 to April 2000

MATERIALS AND METHODS

Three groups of subjects were studied

Drugs given :

- (a) Mefloquine 100 mg to 1500 mg for treatment of malaria
 - (b) Mefloquine 250 mg weekly as a prophylaxis in addition to the usual sulphadoxine-pyrimethamine 1 tablet weekly
 - (c) Sulphadoxine-pyrimethamine 1 tablet weekly
- were included in the study.

MephaquinTM Lactab 250 mg (Mepha Ltd., Aesch Basel, Switzerland)
Pyrixine Tab (Sulphadoxine 500 mg + Pyrimethamine 25mg) (Myanmar Pharmaceutical Factory)

Inclusion criteria

All males, age between 15-55 years who are in one of the above three groups.

Exclusion criteria

Concomitant known illnesses.

Ethical consideration

Ethical clearance was obtained from the Ethical Committee, Department of Medical Research (Lower Myanmar), Ministry of Health.

Procedure

A proforma containing a carefully prepared checklist of neurological psychiatric and non-neurological symptoms including all systems was prepared. A questionnaire was constructed and pre-tested at the CRU (Malaria), DSGH. The study team and trained Medical Officers filled out the questionnaires carefully and completely.

Data analysis

Computer using EPI-INFO software did statistical evaluation.

RESULTS

A total of 2243 cases were obtained.

- (a) 1081 uncomplicated malaria patients on mefloquine therapy (1000-1500 mg) dose
- (b) 249 subjects on mefloquine (250 mg) plus sulphadoxine-pyrimethamine (1 tablet) weekly prophylaxis for 6 months and
- (c) a control group of 913 subjects on sulphadoxine-pyrimethamine (1 tablet) weekly prophylaxis alone for at least 6 months.

Table 1 shows that there was no statistically significant difference between ages and services in three groups.

Table 1. Base line characteristics in three groups

Parameters	Mefloquine Therapy	Mefloquine & Sulphadoxine/Pyrimethamine Chemo-prophylaxis	Sulphadoxine/Pyrimethamine Chemo-prophylaxis
Age (years)	25.6	28.2	26.8
Mean (SD)	(8.96)	(8.06)	(8.53)
Service (years)	7.35	7.52	8.96
Mean (SD)	(7.49)	(7.71)	(7.17)

P > 0.5 between all groups

Table 2. Percentage of patients with or without adverse symptoms in mefloquine therapy group (n=1081)

	No symptoms (%)	With symptoms (%)
Within 24 hours after treatment	23	77
Between 2 to 7 days	12	88
Between 8 to 28 days	96	4

Table 3. Percentage of patients with different groups of adverse symptoms with mefloquine therapy (among symptomatic patients)

	With neuro-psychiatric symptoms (%)	With non-neuropsychiatric symptoms (%)	With both neuro-psychiatric and nonneuro-psychiatric symptoms (%)
Within 24 hours after treatment	43	5	52
Between 2 to 7 days	59	7	34
Between 8 to 28 days	52	24	24

Table 2 and 3 shows that high percentages of symptomatic patients were noted after mefloquine therapy and neuro-psychiatric symptoms were more frequently observed.

The symptoms were transient; majority disappeared within one week and needed no treatment.

Table 4. Commonest neuro-psychiatric symptoms in mefloquine therapy group (n=1081)

	Within 24 hours after treatment	Between 2 to 7 days	Between 8 to 28 days
Dizziness (%)	58	61	1
Insomnia (%)	37	22	1
Headache (%)	33	10	0.3
Tinnitus (%)	32	13	0.2

In patients with neuro-psychiatric symptoms in mefloquine therapy group, majority had dizziness, insomnia, headache and tinnitus (Table 4). All symptoms were mild, transient and self-limiting.

Table 5. Commonest neuro-psychiatric symptoms in mefloquine and sulphadoxine/pyrimethamine chemoprophylaxis group (n=249)

	Within 24 hours after taking drug	Between 2 to 7 days
Dizziness (%)	18.2	2.8
Tinnitus (%)	3.2	0.4
Insomnia (%)	0.8	-
Headache (%)	0.8	-

Table 6. Commonest neuro-psychiatric symptoms in sulphadoxine/pyrimethamine chemoprophylaxis group (n=913)

	Within 24 hours after taking drug	Between 2 to 7 days
Dizziness (%)	5.4	4.6
Insomnia (%)	4.6	2.8
Headache (%)	2.7	1.8
Tinnitus (%)	2.3	3.3

In patients with neuro-psychiatric symptoms in mefloquine and sulphadoxine/pyrimethamine chemoprophylaxis group, only mild symptoms like dizziness were

observed in less than 20% of cases and recovered spontaneously within one week (table 5).

In patients with neuro-psychiatric symptoms in sulphadoxine/pyrimethamine chemoprophylaxis group, dizziness, insomnia, headache and tinnitus were observed in less than 10% of cases (Table 6). All symptoms were mild transient and self-limiting.

Table 7. Commonest non-neuropsychiatric symptoms in mefloquine therapy group (n=1081)

	Within 24 hours after treatment	Between 2 to 7 days	Between 8 to 28 days
Fatigue (%)	38.9	15.6	0.4
Nausea (%)	33.2	7.6	-
Vomiting (%)	28.6	7.4	-
Palpitation (%)	26.8	4.5	-

In patients with non-neuropsychiatric symptoms in mefloquine therapy group, majority had various symptoms like fatigue, nausea, vomiting, palpitation etc. involving many systems. All symptoms were mild, transient and disappeared spontaneously after one week (Table 7).

Non-neuropsychiatric symptoms in mefloquine plus sulphadoxine/pyrimethamine chemo-prophylaxis group and sulphadoxine/pyrimethamine single chemoprophylaxis group were unremarkable and negligible.

In general, no severe neuro-psychiatric or non-neuropsychiatric features were detected in both therapeutic as well as prophylactic groups with mefloquine and also in the control group.

DISCUSSION

This study was done to look specifically for adverse effects related to mefloquine

therapy and prophylaxis. Many symptoms were picked up because of the detailed check-list – enquiring specifically into every system and possible symptom. In fact it was like giving leading questions. They would not have been complained of, if the questionnaire were made in a more subjective open type. It can also be argued that all the symptoms may not be the adverse effects of the drug but the features of the underlying illness or some other concomitant cause.

In this study 1330 subjects received mefloquine (1081 as therapeutic agent 1000-1500 mg and 249 as prophylaxis 250 mg weekly for at least 6 months). The cases were followed up for a period of 4 weeks and some were interviewed again at 3 months. Therefore even if all symptoms picked up in the objective, structured questionnaires were attributed to mefloquine it was found that there were no severe or persistent neuro-psychiatric manifestations.

CONCLUSION

Mefloquine (MephaquinTM – MEPHA) in this study was not associated with serious adverse neuro-psychiatric effects in patients treated with therapeutic dosage of 1000-1500 mg or prophylactic dosage of 250 mg weekly for 6 months. Dizziness, insomnia, headache and tinnitus were recognized as frequent but transient adverse effects of the drug. Coordinated psycho-motor activities were not affected by mefloquine prophylaxis. However, according to WHO recommendations mefloquine should not be used in persons undertaking fine co-ordination and spatial discrimination of air crew.

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Effect of aerobic exercise on body composition and physical fitness

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The effect of aerobic exercise was studied on 20 Myanmar adult sedentary workers. They were engaged in aerobic exercise training of one and half hours per day, five days per week for six weeks. Their exercise intensity was at about 60 to 70 percent of maximum heart rate. The body composition and physical fitness were measured before and after six weeks training. Significant positive increment was found in cardiovascular fitness measured as physical working capacity at heart rate 170 (PWC₁₇₀) (432.11 ± 65.09 kpm/min to 531.84 ± 82.94 kpm/min) and recovery heart rate percent (65.77 ± 6.10 % to 71.19 ± 0.09 %). Lower limbs muscle strength (measured as vertical jumping power) was also significantly increased (31.70 ± 3.56 cm to 34.30 ± 3.16 cm). Although there was not statistically significant, increment was noted in right hand-grip (27.78 ± 3.74 kg to 28.72 ± 3.75 kg) and left hand-grip (24.61 ± 3.53 kg to 25.94 ± 3.73 kg). Body weight (48.49 ± 4.95 kg to 49.47 ± 4.59 kg), lean body mass (36.79 ± 3.19 kg to 36.9 ± 2.80 kg) and fat percentage (11.7 ± 2.50 % to 12.49 ± 2.40 %) were also found to be increased but statistically not significant. This study clearly showed that aerobic exercise can improve physical fitness significantly.

INTRODUCTION

Health related physical fitness means the physical well-being concerning with some aspect of good health and/or disease prevention. The four components of health related physical fitness most commonly evaluated are aerobic or cardiovascular fitness, body composition, muscular strength & endurance and flexibility. A person's performance in each test can be significantly improved through a program of regular exercise and weight control. The finding of thirty years research relating level of physical activity and physical fitness to selected chronic diseases or medical conditions showed clearly that there is a strong association between regular physical

activity, physical fitness and reduction in the risk of dying from heart disease, cancer and other causes [1]. The studies of Finnish men demonstrated that leisure-time physical activity and aerobic capacity had an inverse, graded and independent association with the risk of acute myocardial infarction [2]. For large groups of middle-aged British men, regular physical activity also was protective from heart attack and stroke [3]. In these Finnish and British studies, engaging in just light to moderate regular exercise provided significant protection. The sedentary person is almost twice as likely to develop heart disease as the most active individual. Regarding weight control, in the past, it was generally accepted that the obese condition was the result of an excessive food intake.

Thus, the effective approach to weight loss would be some form of caloric restriction, that is, dieting. In United States, the caloric intake per person has steadily decreased over the past 100 years, yet body mass and body fat has increased steadily [4]. The caloric intake of obese high-school girls and boys is actually below that of their non-obese peers [5]. It is increasingly clear that men and women with a physically active life style or those who become involved in endurance exercise programs tend to maintain a desirable body composition. Evidence is accumulating to support the contention that an increased level of regular physical activity may be more effect than dieting for long-term weight control [6]. Two arguments have been raised against the exercise approach to weight loss. One is the belief that exercise inevitably causes an increase in appetite so that there will be a proportionate increase in food intake. The second argument is that the caloric burning effects of a normal bout of exercise are so small that they will not "dent" the body's fat reserves as significantly as starvation or semi starvation. Therefore, the present study was conducted to assess the effect of 6 weeks basic aerobic exercise regimen on body composition and physical fitness of middle aged female volunteers who were not under dietary control.

MATERIALS AND METHODS

Twenty apparently healthy first and second year nursing diploma female students from the Institute of Nursing, between the ages of 20-40 who were willing to participate in aerobic training course were included. The students who had neither attended any physical training course in the past nor engaged in any sports and regular exercise activity and those with only daily activity of the students were selected. Clinical examination was carried out to exclude anemia, heart diseases, respiratory diseases,

diabetes mellitus and musculoskeletal disorders.

The trainers for the 6 weeks basic aerobic exercise-training course were from the Department of Sports and Physical Education, Myanmar.

The following measurements were done before and after the 6 weeks basic aerobic training course:

Body height was measured by a stadiometer up to nearest 0.1 cm.

Body weight was measured by calibrated Stathmos weighing machine in kg up to nearest 0.1kg.

The limb's circumferences were measured by a flexible steel measuring tape up to nearest 0.1cm.

The skin fold thickness at biceps, triceps, sub scapular and supra iliac, abdominal, thigh and calf sites were measure by Harpenden caliper up to nearest 0.1mm. Body density was calculated according to the formula described by Durnins and Rahaman [7] and body fat % was calculated base on the formula given by Siri [8].

Muscle strength of upper limbs was measured by handgrip and back strength dynamometers. Vertical jumping power was measured by using Sargent's jump-and-reach test.

Flexibility was tested by toe touching, abdominal stretch, twist and touch.

Pulmonary function tests such as vital capacity and FEV₁ were measured by Modular lung analyzer.

Cardiovascular fitness was assessed by PWC170 method using Monark bicycle ergo meter.

The whole training course was conducted for one and half hours per day, five days per week for six weeks.

Statistical analysis

Differences between means of parameters were analyzed by paired 't' test. Significant level was taken as 'p' value less than 0.05.

RESULTS

Table 1 shows general characteristics of the subjects.

Table 1. General characteristics of the subjects

Age (yr.)	21.6 $\pm$ 3.2
Sex	Female
Height (m)	1.55 $\pm$ 0.05
Body weight (kg.)	48.49 $\pm$ 4.95
Body fat %	11.7 $\pm$ 2.50
Education	First and Second year diploma students from University of Nursing
Past physical activity history	No history of attending any physical training course as well as participating sports activity.
Present physical activity history	Not engaged in any type of sports activity.

Table 2 shows comparison of physical fitness before and after 6 weeks aerobic exercise training. Physical performance at heart rate 170 (PWC₁₇₀) and recovery heart rate percent were found to be significantly increased after the training course. Although Vital Capacity (VC) and FEV₁ did not change significantly, the ratio of FEV₁ and VC was significantly improved after the training course. Handgrips and vertical jumping power were found to be increased after the training course. Flexibility test score became significantly greater after the training course.

Table 2. Comparison of physical fitness before and after 6 weeks aerobic exercise training. (n = 18)

	Before	After	't' value	'p' value
Cardiovascular fitness				
PWC ₁₇₀ (kpm/min)	432.11 $\pm$ 65.09	531.84 $\pm$ 82.94	12.03	< 0.00
Recovery heart rate (%)	65.77 $\pm$ 6.10	71.19 $\pm$ 9.09	6.30	< 0.01
Pulmonary function tests				
Vital capacity (L)	2.67 $\pm$ 0.35	2.63 $\pm$ 0.26	1.17	> 0.1
FEV ₁ (L/1 st sec.)	2.49 $\pm$ 0.31	2.53 $\pm$ 0.28	1.22	> 0.1
FEV ₁ / Vital capacity (%)	94.27 $\pm$ 3.97	96.04 $\pm$ 4.55	3.73	< 0.01
Right hand grip (kg)	27.78 $\pm$ 3.74	28.72 $\pm$ 3.75	0.48	> 0.5
Left hand grip (kg)	24.61 $\pm$ 3.53	25.94 $\pm$ 3.73	1.10	> 0.1
Vertical jump (cm)	31.67 $\pm$ 3.56	34.33 $\pm$ 3.16	2.64	< 0.02
Flexibility-				
Toe touching (cm)	34.44 $\pm$ 5.89	42.89 $\pm$ 5.63	4.40	< 0.01
Abdominal stretch (cm)	50.61 $\pm$ 6.71	62.50 $\pm$ 4.29	6.33	< 0.01
Twitch & Touch (cm)	33.61 $\pm$ 14.50	58.89 $\pm$ 14.18	5.29	< 0.01

Table 3 shows change in body composition after 6 weeks aerobic exercise training. There was found to be significant increase in body weight, lean body mass as well as fat percentage.

Figure 1 shows comparison of heart rate response to same load before and after 6 weeks aerobic exercise training. It was found that after the training course, the subjects were able to do same workload with lower heart rate than before.

Table 3. Change in body composition after 6 weeks aerobic exercise training. (n=20)

	Before	After	't' value	'p' value
Body weight (kg)	48.49 ± 4.95	49.47 ± 4.59	0.65	> 0.5
Body fat (%)	11.70 ± 2.50	12.49 ± 2.40	1.02	> 0.1
Lean body mass (kg)	36.79 ± 3.19	36.91 ± 2.80	0.13	> 0.5

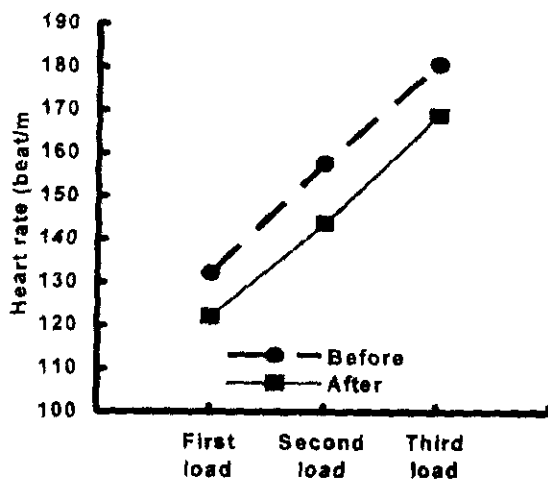


Figure 1. Comparison of heart rate response to same load before and after 6 weeks aerobic exercise training. (n=19).

DISCUSSION

Continuous moderate to high intensity exercise could be effective for weight reduction. Thirty minutes of moderately strenuous running, bicycling, circuit resistance exercise or swimming or at least 60 minutes of walking will stimulate fat loss [9]. The greater the caloric expenditure, the greater the potential will be for body fat loss. This effect is independent of mode of exercise as long as there is a sufficient caloric deficit caused by the exercise. King and Katch reported in 1986 that training at least 3 days per week is required and more frequent training may be even more effective [10, 11]. There had been shown by

Wilmore *et al.* in 1970 that significant weight reduction in body fat percent in 33 men, aged 17 to 59 who jogged 3 days per week for 10 weeks. The average distance run was about 2.8 kilometers per day [12]. It was also found that there was significant decrease in body fat percent, fat folds and waist girth in 3 groups of men (14, 17 and 12 men in each group) who had been trained walking and running for either 15, 30 and 45 minutes per work out respectively for 12 weeks [13]. The two years calisthenics and jogging program could also significantly alter the physique (fat percent, some of fat folds and girth measurements) in 7 previously sedentary 40-60 years old men along with 25 percent improvement in aerobic capacity [14]. In the present study, the subjects were engaged in aerobic exercise regimen of the Department of Sports and Physical Education, one and half hours per day, five days per week for six weeks. Their exercise intensity was about 60 to 70% of maximum heart rate. However, they gained in weight not only in lean body mass but also in fat mass. This may be due to the fact that this type of aerobic exercise for 6 weeks duration without dietary restriction may be inadequate to reduce the fat content of the body.

Regarding cardiovascular fitness, the effect of the training depends upon intensity, frequency and duration of exercise as well as on the initial fitness level of each individual. Most studies recommended that high intensity training (i.e up to or above 70% of maximum heart rate) is necessary in order to gain cardiovascular improvement [15]. Generally the training sessions persist for 20 to 60 minutes, 3 to 5 times each week for several weeks [16]. Some studies showed improvement within 4 weeks [15] and even within 10 days [17, 18]. In the present study, there was significant increase in physical work capacity at heart rate 170 (PWC₁₇₀). It was also confirmed by the following facts. There was significant increase in recovery heart rate percentage (5

minute after exercise) and the subjects were able to do same workload with reduced heart rate than before (Table 2). In the case of pulmonary function, although vital capacity and FEV₁ did not change significantly, the ratio of FEV₁ and VC was found to be significantly increased after 6 weeks aerobic training course. Muscle strength (hand grips and vertical jumping power) and flexibility were also found to be significantly increased. Therefore, it was evident that overall physical performance capacity was significantly increased by this 6 weeks aerobic training regimen.

All subjects were able to participate throughout the study and no untoward physical or mental reaction developing ill health was discovered among them.

From this study, it is shown that six weeks aerobic exercise regimen can improve physical fitness significantly. No untoward injury was observed due to aerobic exercise.

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Isolation of bacterial pathogens from blood samples collected from in-patients, Medical Ward, Defence Service General Hospital, Mingaladon during 1998-1999

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By using a random convenience sampling method, culture was done on 81 blood samples of patients with high fever (including septicaemia, pyrexia of unknown origin, enteric fever, malaria etc.) from September, 1998 to April, 1999 at Defence Service General Hospital, Mingaladon. The samples were randomly collected from 57 males and 24 females (age ranges from 13 to 78 years). Bacterial pathogens were isolated from 28 cases (34.57%), either aerobically and/or anaerobically. Of these, six patients resulted in death (8.33%). Bacterial species included: *Diplococcus* species (3.57%); *Escherichia coli* (17.86%); *Klebsiella aerogenes* (7.14%); *Pseudomonas pyocyanea* (17.85%); *Proteus morganii* (14.29%); *Staphylococcus aureus* (28.57%); *Streptococcus faecalis* (3.57%) and gram variable organisms (3.57%). They were resistant to ampicillin (85.71%); chloramphenicol (61.91%); cefalothin (57.14%); furazolidone (23.81%); gentamicin (28.57%); streptomycin (85.71%); septrin (52.38%); and tetracycline (52.38%). However, they were sensitive to amikacin (95.00%) and norfloxacin (95.24%); gentamicin (57.14%) and netilmicin (80.95%).

INTRODUCTION

Septicaemia is defined as a clinical syndrome characterised by signs of overwhelming infection including haemodynamic change. The terms sepsis and septicaemia are used somewhat loosely, but generally refer to the clinical syndrome complexes associated with bacteraemia. They include fever, chills, hypotension, shock, which indicate the evidence of spread of the pathogens to various organs. The clinical findings may develop acutely, as in gram-negative septic shock, or slowly, as in most forms of infective endocarditis. Bacteremia in Bangladesh was reported; the most common pathogens isolated were Enterobacteriaceae, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other non-glucose-fermenting bacilli, *Streptococcus pneumoniae* and *Haemophilus influenzae* [1].

Septicaemia is often a life-threatening and fatal condition which necessitates immediate initiation of treatment. For accurate diagnosis and appropriate choice of antibiotics, blood culture is required and this usually takes a few days. Thus, empirical choice of antibiotics in the treatment of bacteraemia by clinicians, would be guided by the knowledge of previous culture report. The updated information on the local aetiological patterns and antibiotic sensitivities of the blood isolates needs to be explored in this area.

Therefore, the present study is conducted to obtain some information on the frequency of blood culture isolates from septicaemia cases admitted in Defence Services General Hospital. Antibiotic susceptibility tests were performed on the isolated blood cultured samples.

MATERIALS AND METHODS

Selection of subjects

Patients with fever for at least two days or more, irrespective of the admitting diagnosis, during September 1998 to April 1999 were chosen by random convenience sampling method. A total of 81 samples (57 males and 24 females) were obtained for this study. Their age group ranged from 13 to 78 years.

Primary inoculation

Blood samples were collected from patients and inoculated in blood culture bottles, containing 50 ml of tryptose phosphate broth with 0.025 percent polyanethol sulfonate (liquoid) and thioglycollate media. They were incubated aerobically and anaerobically at 37°C for 10 days and checked daily for turbidity as an indication of growth.

Isolation and identification

Based on the gram staining characteristics of the bacteria, growth in the blood culture bottles were subcultured onto MacConkey agar (MA), Blood agar (BA), Mannitol Salt Agar (MSA), Nutrient Agar (NA); Salmonella-Shigella (SS) agar and Thiosulphate Citrate Bile Sucrose (TCBS) agar plates. After incubation, colonies were further characterised biochemically, and then by serology [2].

Antibiotic susceptibility tests

The susceptibility status of all isolates were tested using antibiotic discs from BBL (USA); Oxoid (UK); and Hi (India) according to the method of Bauer, Kirby, Sherris and Turck, 1966 [3].

For gram-negative isolates, the antibiotic discs as: amikacin, ampicillin, augmentin, cephalothin, chloramphenicol, furazolidone, gentamicin, kanamycin, septrin, sisomicin, streptomycin and tetracycline were used while, augmentin, cephalothin, chloramphenicol,

erythromycin, gentamycin, lincomycin, methicillin, nalidixic acid, penicillin, streptomycin and tetracycline discs were used for gram positive organisms.

RESULTS

Bacterial pathogens isolation

Bacterial species isolated from septicaemia cases are shown in Table 1.

Table 1. Bacterial pathogens isolation from septicaemic cases with age and sex

Age group years	Number of cases			
	Male	Female	Pathogens	Deaths
13-20	16	5	8 (38.09)	5 (23.81)
21-30	10	10	6 (30.00)	0
31-40	8	2	2 (20.00)	0
41-50	10	1	6 (54.55)	0
51-60	9	4	6 (46.15)	0
61>	4	2	0	1 (25.00)
Total	57 (70.37)	24 (29.63)	28 (34.57)	6 (8.33)

Figures in parenthesis denote percentages

A total of 81 patients, (male: 70.37% and females: 29.63%) were included in this study. Out of 81 septicaemic patients, bacterial pathogens were isolated from 28 cases (34.57%). These include 8 cases (38.09%) from the age group of 13-20 years: *Pseudomonas pyocyanea*, *Proteus morganii*, *Staphylococcus aureus*, and *Diplococcus* species were isolated from 4,2,1, 1 cases respectively. In this age group, five cases (23.81%) were died during the study period. Out of 6 cases (30%) from the age group of 21-30 years, *Staphylococcus aureus*, *Proteus morganii*; *Klebsiella aerogenes* and *Streptococcus* species were isolated from 2, 2, 1, 1 case respectively. In the age group of 31-40 years, *Staphylococcus aureus* and *Escherichia coli* were isolated from one case

each. In the age group of 41-50 years, *Escherichia coli*, *Pseudomonas pyocyanea*, *Streptococcus faecalis* and *Staphylococcus epidermidis* were isolated from 3, 1, 1, & 1 case respectively. In the age group of 51-60 years, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes* and gram variable were isolated from 3, 1, 1, & 1 case respectively.

Bacterial species isolated from septicaemic cases

Out of 28 cases, the isolated bacterial pathogens were as following: *Diplococcus* species (from 1 case: 3.57%); *Escherichia coli* (6 cases: 21.42%); *Klebsiella aerogenes* (2 cases: 7.14%); *Pseudomonas pyocyanea* (5 cases: 17.85%); *Proteus morganii* (4 cases: 14.29%); *Staphylococcus aureus* (8 cases: 28.57%), *Streptococcus faecalis* (1 case: 3.57%) and gram variable (1 case: 3.57%) (Table 2).

Table 2. Isolated bacterial species from septicaemic cases

Sr. No	Bacterial species	Aero-bic	Anae-robic	Aero-bic & Anae-robic	Total
1	<i>Diplococcus</i> spp.	1	0	0	1 (3.57)
2	<i>Escherichia coli</i>	2	3	1	6 (21.43)
3	<i>Klebsiella aerogenes</i>	2	0	0	2 (7.14)
4	<i>Pseudomonas pyocyanea</i>	2	0	3	5 (17.85)
5	<i>Proteus morganii</i>	1	1	2	4 (14.29)
6	<i>Staphylococcus aureus</i>	1	1	6	8 (28.57)
7	<i>Streptococcus faecalis</i>	1	0	0	1 (3.57)
8	Gram variable	1	0	0	1 (3.57)
Total		11	5	12	28
		39.29	17.86	42.86	100.00

Figures in parenthesis denote percentages

Bacterial pathogens isolated from the study population

Irrespective of diagnosis on admission, bacterial pathogens isolation from the study population was shown in Table 3.

Table 3. Association of bacterial pathogens isolation with the diagnosis on admission

Diagnosis on admission (or) Provisional diagnosis	tested	Patho-gens isolated	Types of pathogens
Septicaemia	11	8 (72.73)	<i>Escherichia coli</i> : 2 <i>S. aureus</i> : 2 <i>Ps. pyocyanea</i> : 2 <i>Proteus morganii</i> : 2
P. U. O	4	3 (75.00)	<i>S. aureus</i> : 1 <i>S. pyogenes</i> : 1 <i>Pseudomonas pyocyanea</i> : 1
Enteric fever	13	3 (23.08)	<i>S. aureus</i> : 2 <i>S. faecalis</i> : 1
Pneumonia/ chest infection	11	3 (27.27)	<i>Diplococcus</i> spp.: 1 <i>Escherichia coli</i> : 1 Gram variable: 1
Endocarditis/ myocarditis	6	3 (50.00)	<i>Escherichia coli</i> : 1 <i>Ps. pyocyanea</i> : 1 <i>S. aureus</i> : 1
Liver abscess /cirrhosis	5	2 (40.00)	<i>Klebsiella aerogenes</i> : 1 <i>Ps. pyocyanea</i> : 1
Meningitis/ brain abscess	4	Nil	
Leptospirosis	3	1 (33.33)	<i>S. aureus</i> : 1
Septic abortion	1	Nil	
Japanese encephalitis	2	Nil	
Urinary tract infections	2	1 (50.00)	<i>Escherichia coli</i> : 1
Others	11	2 (18.18)	<i>Escherichia coli</i> : 1 <i>S. epidermidis</i> : 1
Malaria	8	2 (25.00)	<i>Klebsiella aerogenes</i> : 1 <i>Proteus morganii</i> : 1
Total	81	28 (34.57)	

Figures in parenthesis denote percentages

Escherichia coli, *Staphylococcus aureus*, *Pseudomonas pyocyanea* and *Proteus morganii* were isolated from 72.73% of septicaemia cases. *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas pyocyanea* were isolated from PUO. The bacterial pathogens were isolated from 23.08%, 27.27% and 50% of the cases of enteric fever, chest infection and heart diseases respectively.

DISCUSSION

Bacteraemia could be associated with many human infections and usually occurs in the early stages of illness. The organisms isolation from the blood is undertaken less frequently. If the causal bacterial pathogen could be isolated and identified from the blood culture, it would give a definite early diagnosis of the illness. As for an example, if the *Salmonellae* spp. could be isolated from PUO cases in blood culture samples during the first week of the illness, it gives the definite diagnosis of that case. In this study, bacterial pathogens were isolated from 34.57% of the cases. The low isolation rate might be due to the inhibition of antibiotics that have been taken by these patients before admission. The isolated *E. coli* strains were not agglutinated by the 24 available antisera in this study. Therefore, other toxin assay (verotoxin, haemolysin) and cell damage activity test need to be explored. In this study, the isolated strains were resistant to ampicillin and streptomycin (85.71%), chloramphenicol (61.91%), cephalothin (57.14%), tetracycline and septrin (52.38%). However, these strains were sensitive to norfloxacin (95.24%), amikacin (95.00%), netilmicin (80.95%), and gentamicin (57.14%). The importance of bacterial pathogens isolated from blood culture [4-10] and emergence of antibiotic resistance during therapy was well documented by various authors [11-12]. Although knowledge of the sensitivity of bacterial strains cannot be the sole factor for rational prophylactic or therapeutic use of

antibiotics, it is certainly essential for the appropriate choice of antibiotics. This report thus serve as an important and useful information in the aetiology of septicaemia and drug susceptibility pattern for the management of these patients.

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***In vivo* study of the prophylactic value of some plants against experimentally-induced infection of closed and open wounds**

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Coptis teeta, (khan-tauk), *Lawsonia alba* (dan-gyi), *Quisqualis indica* (dawe-hmaing), and *Stephania hernandifolia* (taung-kya-kyet-thway) were tested for *in vivo* closed wound infected with *Staphylococcus aureus*. Viable bacterial count, tensile strength and tissue collagen content were measured and compared with three control groups, one with paraffin alone treatment, another with sterile gauze alone and with classical drug tetracycline ointment. The most promising results were observed with extracts of *C. teeta* and *S. hernandifolia* than the remaining two plants. As for open wounds, linear open wounds infected with *S. aureus* in albino rats treated with *Kaempferia* spp. (bulb powder, benzene extract, and 50% alcoholic extract) and kanamycin, distilled water, sulphanilamide, talcum powder and no treatment were used as controls. Out of various treatments, the *Kaempferia* spp. powder in paraffin gave the most promising results.

INTRODUCTION

The formation and prevention of wound infections involve many factors in addition to bacterial contamination. Since the basis of all wound infections is bacterial, infection could theoretically be eliminated if the organism could be totally eradicated. Reports from workers elsewhere also bear that *Staphylococcal* disease is a problem and the serious nature of *Staphylococcal* wound infections is well documented [1-3]. In Ayurveda medicine, the types of wounds and their management has been elaborated in great detail by the pioneer surgeon Sushruta. In his treatise, 60 procedures in the managements of wound have been described and has enumerated a large number of herbal drugs to be used in the management of wounds singly or in combination, in different forms, such as juice, decoctions, and ointments. Wound healing activity of various plants were also recorded such as *Garcinia morella* [4]; *Helianthus annuus* [5]; *Jasmina auriculatum*

[6]; *Salvadora persica* [7]; *Vernonia cineria* [8]; and *Woodfordia fruticosa* [9]. Thus this paper aims to determine some activity of plants in experimentally induced infected open and closed wounds in rats.

MATERIALS AND METHODS

Experimental closed wound model

The experiment closed wound model was introduced in rats as describe by the method of Kim & Rosenthal [10].

Organisms used in inducing artificial infection

Staphylococcus aureus was used as described by Gingrass *et al.* [11,12].

Tensile wound strength of the wound tissue

Progress of the wound healing with respect to its biomechanical nature was assessed by measuring the tensile strength of tissue from wound area as describe by Deshpande *et al.* [13].

It has been observed that mitotic activity of epithelial cells begins after 48 hrs and attains its peak by 72 hrs. The chief components of the healing wound are collagen fibres and thus measured in terms of hydroxyproline level in the tissue by the method as described by Deshpande *et al.* [14].

Linear open wounds infection

Longitudinal incisions of 40 ± 2 mm in length were made in rats infected with *S. aureus* and assessment of wound healing rate was determined daily by measuring the length of unhealed area by the modified

RESULTS

Effect of plant extracts on the viable bacterial count on closed wound preparation

As shown in Fig. 1, in the control group (non-drug treatment), there was a gradual reduction in the viable bacterial count throughout the experimental period: i.e. 5.2, 3.7 and 3.5 million organisms per gram tissue on post operative day-4, day-8 and day 14 respectively. The effect of control classical drug tetracycline, *C. teeta*, *L. alba*, *Q. indica* and *S. hernandifolia* also caused a marked reduction.

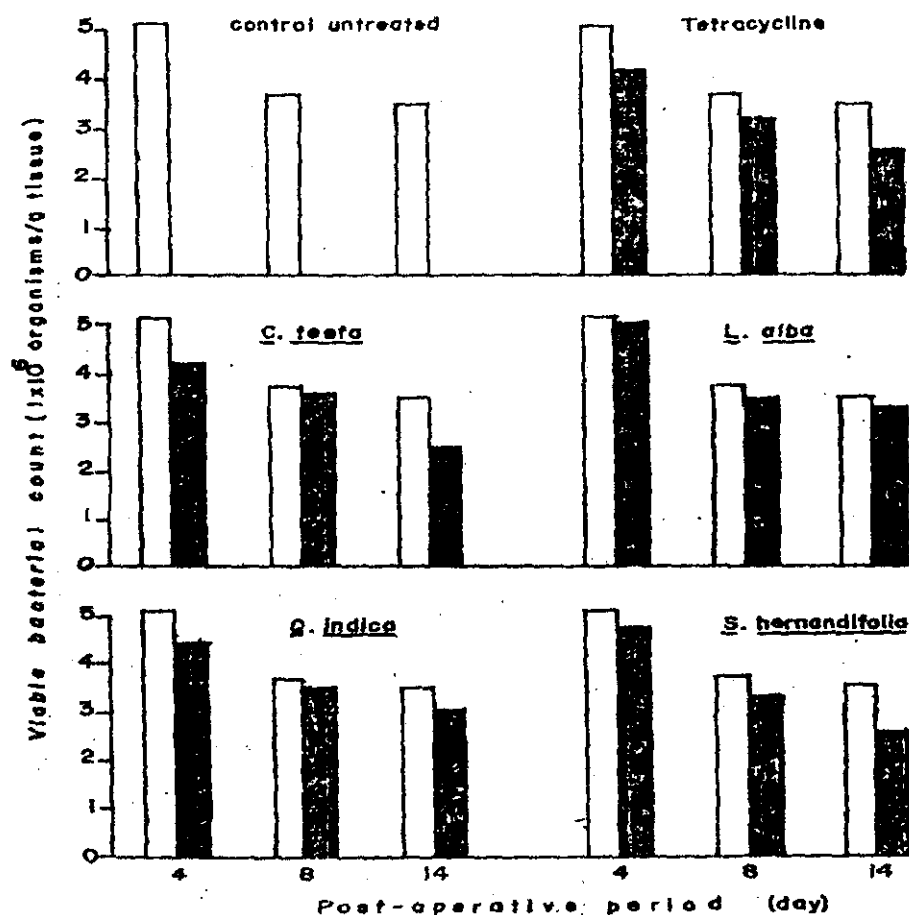


Fig. 1. Effect of plant extracts on the viable bacterial count in experimental infected wounds. Each column denotes the average of 3 to 5 numbers of observations control untreated observations (□); Various drug treatment (■)

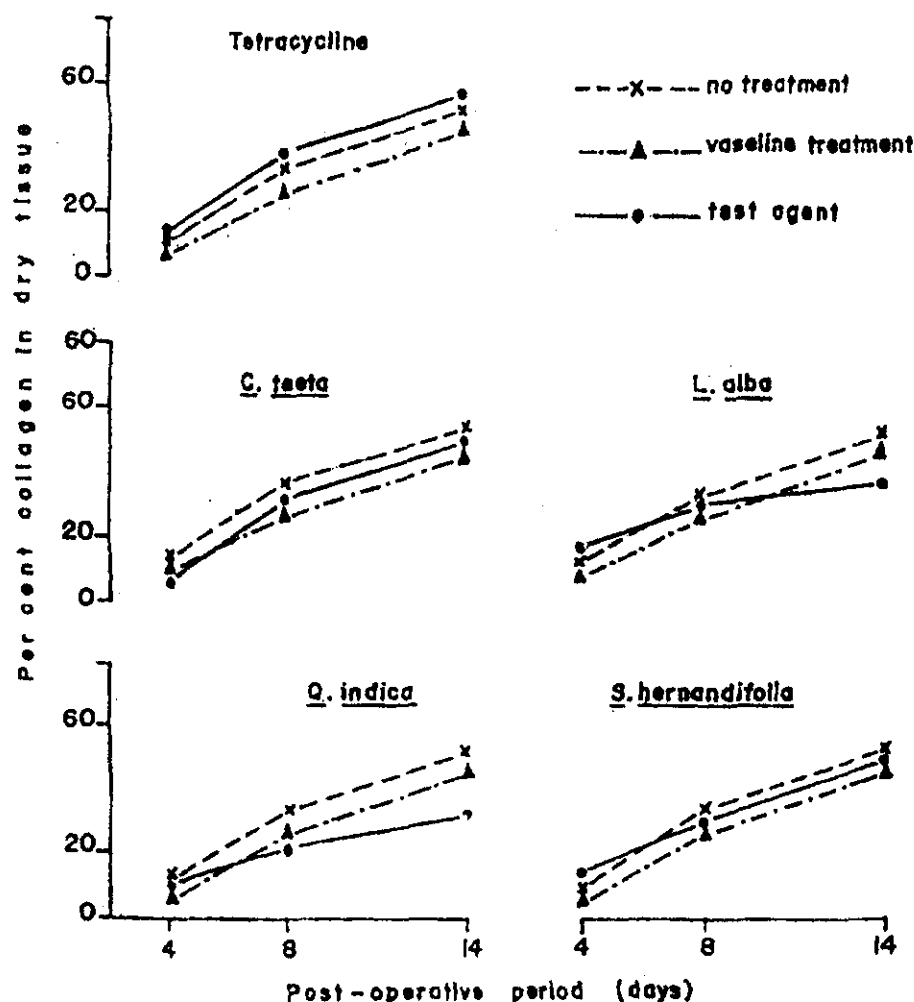


Fig. 2. Effect of *C. teeta*, *L. alba*, *Q. indica*, *S. hernandifolia* and tetracycline on the tissue collagen content of closed wound preparation

Effect of some plant extracts on the tensile strength of closed wound preparation

The time course effects of those investigations are illustrated in Fig. 2. In the group of animals receiving no treatment at all, the tensile strengths were 116g, 136g, and 199g on post-operative day-4, day-8 and day-14 respectively. In the group of animals receiving only the application of vaseline alone the tensile strength were 121g, 143g and 203g at post-operative day-4, day-8 and day 14 respectively. Similar gain in tensile strength was also observed with tetracycline and plants tested with most promising effect with *C. teeta* and *S. hernandifolia* than *L. alba* and *Q. indica*.

Effect of some plant extracts on the tissue collagen content of closed wound preparation

The time course effects of those investigations are shown in Fig. 3. In the group of animals receiving no treatment at all, the tissue collagen content were 7%, 19% and 25% on post operative day-4, day-8 and day-14 respectively. With application of baseline, only it shows 4%, 14% and 24% respectively on post-operative day-4, day-8 and day-14 respectively. Similar gain in tissue collagen content was also observed with tetracycline and plants tested with most promising effect with *C. teeta* and *S. hernandifolia* than *L. alba* and *Q. indica*.

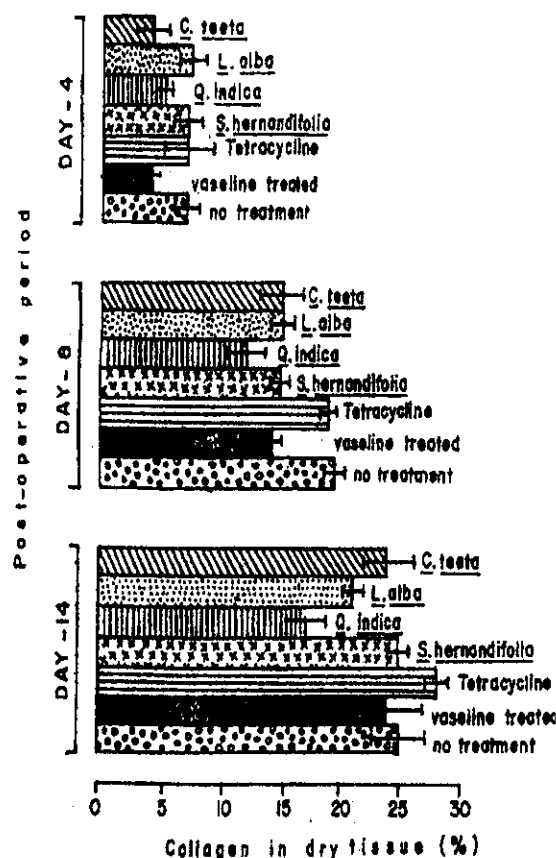


Fig.3. Comparative effect of *C. teeta*, *L. alba*, *Q. Indica*, *S. hernandifolia* and tetracycline on the tissue collagen content of closed wound preparations.

Each bar denotes the average of 9 to 15 number of observations. Horizontal bars indicate standard error of the means.

Effect of various products of *Kaemferia* spp on open wound preparations

The time course of wound healing effects of various products of *Kaemferia* spp. (powder, benzene extract, and 50% alcoholic extract) in comparison with that of distilled water) together with the durations required for complete wound healing are shown in Fig. 4. In the control group, there was a steady decrease in unhealed wound measurements from the initial value of 40.4 ± 0.4 mm to 8.6 ± 2.3 mm on the tenth post-operative day and the wound healed completely in 12 ± 1 days. The *Kaemferia* spp powder in paraffin possessed a better wound healing property apparently beginning on the sixth post-operative day

and markedly better ($p < 0.25$) on the tenth postoperative day when compared with that of control.

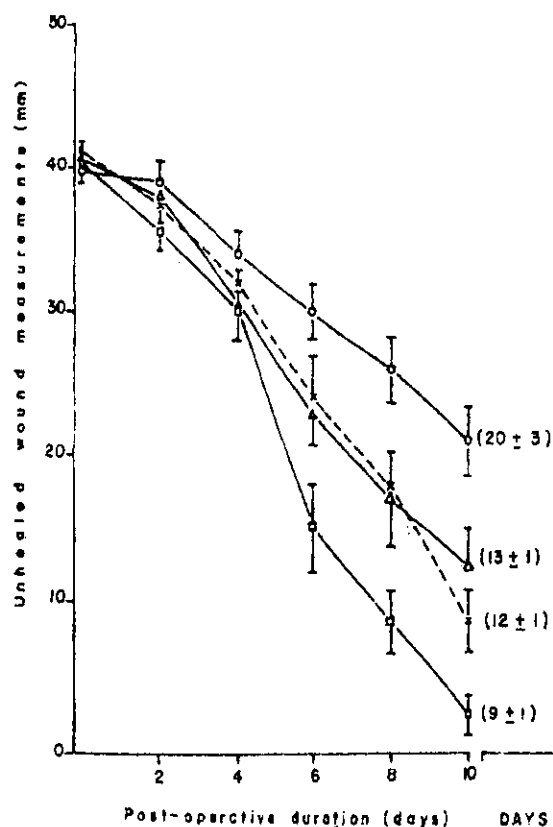


Fig. 4. Effect of various product of *Kaemferia* spp. on open wound preparations

Each point represents the means of 10 to 11 observations for groups treated with various *Kaemferia* spp. products - powder in paraffin —○—, benzene extract —□— and 50 per cent alcoholic extract —△— and 31 observations for the distilled water treated —*— control groups. Vertical bars indicate standard error of the means. Value in the parenthesis indicate the respective average duration (days) required for complete cure with its standard errors of the mean.

The powder form also significantly shortened ($p < 0.125$) the duration of complete wound healing to 9 ± 1 day. In contrast, the benzene extract of *Kaemferia* spp. significantly inhibited ($p < 0.01$) the extent of wound healing throughout the test period and the effect was found markedly less on the tenth day, when compared with

the control group. In addition, the extract markedly prolonged ($p < 0.0095$) the duration of complete wound healing to 20 ± 3 days.

Effect of Kaemferia spp. and kanamycin on open wound preparation

The time course of wound healing effect of *Kaemferia* spp. (benzene extract) and those of kanamycin together with the durations required for complete wound healing are shown in Fig. 5.

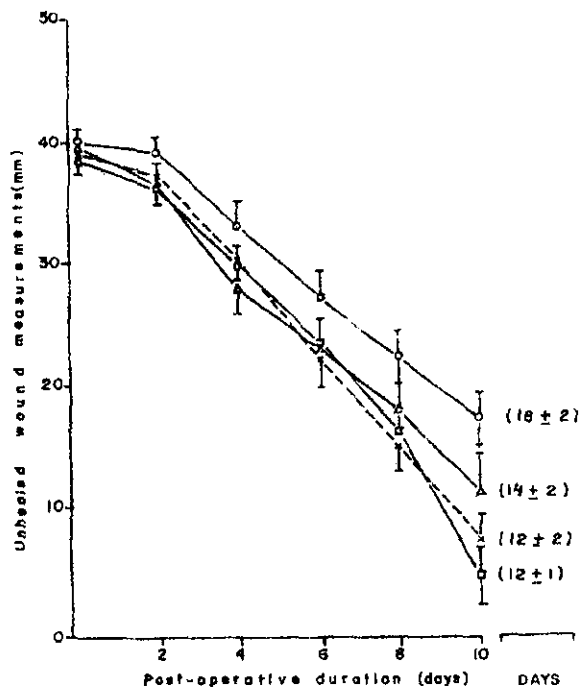


Fig. 5. Effect of *kaemferia* spp. and kanamycin on open wound preparations

Each point represents the means of 10 observations for groups treated with various *Kaemferia* spp., benzene extract —○—, and kanamycin —□— and distilled water —△— and 30 observations for the sterile gauze treatment "control" group —*—. Vertical bars indicate standard error of the means. Value in the parenthesis indicate the respective average duration (days) requires for complete cure, with its standard errors of the means

In the control group (treated only with sterile gauze), there was a decrease in the unhealed wound measurements from the initial value of 39 ± 0.8 mm down to 7.2 ± 1.96 mm on the tenth post-operative day,

and the wound healed completely in 12 ± 2 days. However, the benzene extract of *Kaemferia* spp. apparently inhibited the extent of wound healing which was found to be significantly less on the tenth day when compared with groups even either sterile gauze treatment ($P < 0.01$), or distilled water ($P < 0.01$), or kanamycin treatment ($P < 0.005$). The extract also significantly prolonged ($P < 0.0005$) the duration of complete wound healing to 18 ± 2 days, when compared with that of kanamycin.

DISCUSSION

Many local plants are reputed for their usefulness in the treatment of wounds. Though assessments of wound healing effects are done on different species of animals with incised wounds, which are either infected or not infected, albino rats are the commonest animals used by many workers [16]. Gingrass [11] used 4.6 to 8 million organisms for inoculation of a wound with a median and mean of 5.7 million. In our series 5.9 million organisms per ml with a range of 4.8 to 6.5, million organisms were used. Tensile strength of wounds give quantitative information regarding the repair of healing of wounds. Many methods are devised for measuring the strength of wounds including machines (17). The present work, in addition, is encompassing on both healing activity of the infected wound and the antibacterial activity of test agents. The infected wound model therefore was found to produce consistent results regarding determination of the tensile strength and thereby its applicability in the assessment of the antibacterial agents. Local people in the outlying areas apply *Kaemferia* spp in powder form to their wounds receiving while working in the farms and fields. The wounds encountered were usually from the open categories. The present study assessed the healing of open wounds by noting the contraction of the wound and the duration required to achieve complete wound healing. The powder form in paraffin shows

promotion of wound healing than other forms of application and suggested as usefulness in curing wounds.

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***In vitro* antibacterial activity of some indigenous plants and effect on
in vivo Staphylococcal induced wounds**

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For *in vitro* study, 12 indigenous plant extracts were tested on 5 strains of *Escherichia coli* (EPEC, ETEC, VTEC, EAEC and ATCC), 3 strains of *Shigellae* (*Shigella boydii*, *Shigella dysenteriae*, *Shigella sonnei*); one strain each of *Klebsiella aeruginosa*, *Plesiomonas shigelloides*, *Proteus morganii*, *Pseudomonas pyocyanea*, *Salmonella typhi*, *Staphylococcus aureus*, and *Vibrio cholerae*. The antibacterial activity of plants on tested bacterial species were *Ageratum conyzoides*: hkwathaipan (4 species); *Coleus aromaticus*: ziyawethu (5 species); *Cuminum cyminum*: ziyasai (2 species); *Emblia officinalis* syn. *Phyllanthus emblica*: zibyuthee (11 species); *Foeniculum vulgare*: samonsaba (1 specie); *Nyctanthes arbortristis*: seikphaluywet (6 species); *Piper betle*: kunywet (13 species); *Piper nigrum*: ngayokkaungsai (5 species); *Terminalia chebula*: (11 species); and *Vinea rosea*: thinbawmahnyoywet (6 species) respectively were demonstrated by using agar disc diffusion technique. For *in vivo* study, *Staphylococcus aureus* strain was induced as open wounds in experimental rats and topical application of plant extracts in paraffin was introduced. It was noted that the plant *Piper betle* (kun) and *Nyctanthes arbortristis* (seikphalu) accelerated the rate of wound healing and tensile strength without formation of pus and induration when compared with the controls.

INTRODUCTION

Plants and their products appear to have been used in the treatment of infectious diseases from very long time, even before the discovery of microorganisms. The systematic investigation of higher plants for antibiotic substances was studied by Osborn in 1943 [1] who screened 2300 species from 106 families against *Staphylococcus aureus* and *Escherichia coli*. Since then many investigators surveyed on higher plants from their respective countries to obtain new drugs for chemotherapy. Screening of Indian plants for biological activity was reported since 1968 [2] and recently in 1996 (3). Various investigators also demonstrated the

antibacterial activity of *Heracleum* species (sulana variety) [4]; *Euclyptus maidenii* (yuclyp) [5]; *Allium ursinum* (kyetthun variety) [6]. In Ayurveda medicine many plants were used in different ailments and some leaves were applied to wounds to act as styptic and heal them quickly. Among them *Ageratum conyzoides* (hkwathaipan) was included. Plants possessing wound healing activity which was shown by various authors were *Gaccinia morella* (pannyogyi); *Helianthus annus* (negya); *Hydrocotyl asiatica* (myinkhwa); *Jasmina auriculatum* (zunpan); *Salvadora persica* (local name not known); *Veronia cineria* (kadupyan, thahadevi); and *Woodfordia fruticosa* (panle, pattakyi) [7]. The objective of this

study is to observe *in vitro* testing of *Nyctanthes arbortritis* in parallel with other eleven plants on some human pathogenic bacteria isolated from clinical specimens and to observe the *in vivo* testing of *N. arbortritis* on experimentally induced open wounds infected with *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant extraction

(a) Fifty per cent alcoholic extract

Crushed powder of air dried parts were refluxed in petroleum ether in a soxhlet apparatus to remove chlorophyll and waxy substances. The residue after extracting with petroleum ether was dried and extracted with 50 per cent ethanol. It was then concentrated under reduced pressure at low temperature (38-40°C). The final concentrate thus obtained was dried in a vacuum dessicator.

(b) Fifty per cent alcoholic extract water soluble moiety

The alcoholic substance obtained from above was then eluted with distilled water. The water soluble fraction was then concentrated under reduced pressure and at low temperature and dessicated under reduced pressure at room temperature.

Screening of antibacterial indigeneous plant extracts by agar disc diffusion technique

The different parts and different extracts of the plants tested are listed in Table 1. The organisms used are shown in Table 2. Screening was done by the use of impregnated filter paper discs. The discs, 8 mm in diameter, punched from Number 1 Whatman filter paper were sterilised by autoclaving followed by dry heat at 60°C

for one hour. It was then impregnated with concentrated extracts (aprox. 1 to 2 mg/discs) and then allowed to dry at room temperature. A few colonies (3 to 10 organisms) to be tested were picked with a wire loop from the original culture plate and introduced into a test tube containing five millilitre of Mueller Hinton broth and followed by the method of Bauer, Kirby, Sherris and Turck, 1966 [8].

Table 1. Plant extracts used for antibacterial screening

Sr. No.	Botanical name	Myanmar name	Family	Parts used	Place
1	<i>Ageratum conyzoides</i> L.	Hkwa-thai-pan, Ka du pho	Compositae	Whole plant	MnO, Y
2	<i>Coleus aromaticus</i> Benth.	Zi ya ywet tu, Ya wa ni ywet	Labiatae	Leaf	S, Ind., L
3	<i>Cuminum cyminum</i> L.	Zi ya sai	Umbelliferae	Seed	M
4	<i>Emblica officinalis</i> Gaerth	Zee byu thee	Euphorbiaceae	Fruit	M, C
5	<i>Foeniculum vulgare</i> Gaerth.	Sa mon sa ba	Urticaceae	Leaf	U, V, NM
6	<i>Girardinia zeylonica</i> Dcne.	Nwe tha gi	Apocynaceae	Leaf	L
7	<i>Nerium indicum</i> Mill.	Nwe tha gi	Apocynaceae	Leaf	L
8	<i>Nyctanthes arbortritis</i> L.	Seik phalu	Oleaceae	Leaf	C, Ind
9	<i>Piper betle</i> L.	Kun ywet	Piperaceae	Leaf	L
10	<i>Piper nigrum</i> L.	Nga yok kaung	Piperaceae	Seed	L
11	<i>Terminalia chebula</i> Retz.	Pan ga thee	Combretaceae	Fruit	M, Pgu, T
12	<i>Vinca rosea</i> L.	Thinbaw ma hnyo	Apocynaceae	Leaf	Y, Pma

C. = Chin, Chin hills; Ind. = Indian; L. = Lower Myanmar; M. = Myanmar, widely distributed; Mmo. = Maymyo; NM. = North Myanmar; S. = Sea shore; U. = Upper Myanmar; V. = Valley; Y. = Yangon; Pgu. = Bago; Pma. = Pyinmana; T. = Tanintharyi

Table 2. Bacterial species tested for antibacterial activity

Sr. No.	Test organisms	Source
1	<i>Escherichia coli</i>	ATCC 25922
2	<i>Escherichia coli</i>	EAEC N3/83, DMR
3	<i>Escherichia coli</i>	EPEC 0128, DMR
4	<i>Escherichia coli</i>	ETEC H 1-3, DMR
5	<i>Escherichia coli</i>	VTEC Su Mon 10-1
6	<i>Klebsiella aeruginosa</i>	NCTC-418, Biken, Japan
7	<i>Plesiomonas shigelloides</i>	WT-8
8	<i>Proteus morganii</i>	Biken, Japan
9	<i>Pseudomonas pyocyanea</i>	DTW
10	<i>Salmonella typhi</i>	Biken, Japan
11	<i>Shigella boydii</i>	N 136-7
12	<i>Shigella dysenteriae</i>	Sd-4
13	<i>Shigella sonnei</i>	NO 398-2, DMR
14	<i>Staphylococcus aureus</i>	WS-1
15	<i>Vibrio cholerae, Inaba</i>	KSA, DMR

Evaluation of the healing activity of Staphylococcus aureus induced open wounds (in vivo)

Adult *Rattus norvegicus* rats of both sexes weighing 154 to 220g comprising four to six animals per group were used in the experiments: open wounds to be infected were made, using a modified method of Hopson, Britt, Sherman and Ledes, 1968 [9]. Inoculation of the wounds with *Staphylococcus aureus* was done as described by Gingrass, Close and Ellison, 1964 [10].

Surgical technique of open wound preparation for drug testing

An inhalation anaesthetic diethyl ether was administered to the rat, both for the induction and maintenance of anaesthesia for the operation. The flanks were chosen for the sites of operation. The areas were initially shaved with a razor blade and then sterilised by applying serially with methylated spirit. Longitudinal incisions of three centimeters length, was made in each sterilised area until the depth of incision would cut through some fibers of the muscular layer.

Drugs, chemicals and surgical application used

- (1) Plant parts (extract 50 % water, 50% ethanol)

- (2) Paraffin (BDH chemicals Ltd., Poole, England)
- (3) Sterile gauze (Myanmar Pharmaceutical Industry, Yangon)

Assessment of the wound healing rate

The wounds were daily observed and dressed with either the test drug/drugs or just sterile dressing. Estimation of wound healing was made by measuring the length of unhealed portion of the wound, in cm. At the same time, evidence of induration erythema and the presence of pus were checked and noted. On every alternate day pus obtained prior to the dressing was submitted for bacterial culture.

Effects of the *Nyctanthes arbortristis* L and *Piper betle* L. extract was investigated by comparing the observations obtained from wound of individual animal. The rate of wound healing was determined using two parameters: [1] the average duration required for complete healing of the wound and [2] the time course of the wound healing effect (expressed in terms of measurement of the unhealed wounds) during the first ten post-operative days. Observations and measurements were recorded daily until complete cure was achieved. The tensile strength of open wound preparation after post-operative period of 14 days was also carried out.

The results were presented graphically and analysed statistically. The student's t- test was applied as a test of significance whenever necessary and a significant level of $P < 0.05$ was used, unless otherwise stated.

RESULTS

Effect of indigenous plant extracts (50% ethanolic) on some bacteria

Table 3 represents the antibacterial activity of some plant extracts on tested bacteria.

Table 3. Effect of indigenous plant extracts (50 percent ethanol) on some bacteria. The figures indicate the mean zone diameter in millimeter

Plants	organisms														
	<i>E. coli</i> (ATCC)	<i>E. coli</i> (H1-3)	<i>E. coli</i> EPEC O120	<i>E. coli</i> EAEC N3/83	<i>E. coli</i> (VTEC)	<i>S. boydii</i>	<i>S. dysenteriae</i>	<i>S. sonnei</i>	<i>K. aeruginosa</i>	<i>P. shigelloides</i>	<i>P. morganii</i>	<i>P. pyocyanea</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>V. cholerae</i>
<i>A. geratum conyzoides</i> L.	-	-	-	-	-	12	16	12	-	-	-	-	-	-	17
<i>Coleus aromaticus</i> Benth	-	-	-	-	-	-	14	16	-	-	-	-	20	14	21
<i>Coleus aromaticus</i> ¹ Benth	26	28	40	26	-	48	35	30	-	-	30	-	50	30	40
<i>Cuminum cyminum</i> L.	-	-	-	-	-	-	-	-	-	-	-	-	15	-	18
<i>Emblica officinalis</i> Gaerth	13	12	12	-	-	13	13	13	-	13	13	15	12	17	-
<i>Foeniculum vulgare</i> Gaerth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
<i>Girardinia zeylonica</i> Dene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Nerium indicum</i> Mill.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Nyctanthes arbortristis</i> L.	-	-	-	-	-	17	12	13	-	-	-	-	16	18	21
<i>Piper betle</i> L.	16	16	16	18	16	NT	17	15	15	17	16	-	19	15	20
<i>Piper nigrum</i> L.	-	-	-	-	-	16	-	12	-	-	-	-	14	16	20
<i>Piper nigrum</i> ¹ L.	-	-	-	-	-	-	12	14	-	-	-	-	18	16	20
<i>Terminalia chebula</i> Retz	-	12	12	14	-	15	15	15	12	-	15	12	12	19	-
<i>Vinca rosea</i> L.	-	-	-	-	-	16	13	12	-	-	-	-	18	13	17

¹ essential oil

The zone of inhibition in millimeter was shown. *Coleus aromaticus*, *Emblica officinalis*, *Piper betle* and *Terminalia chebula* were found to be more active against the tested bacteria than other plants tested.

Effect of *Piper betle* and *Nyctanthes arbortristis* on experimentally induced wounds by topical application

In Fig. 1 the effect of the two plants on open wound preparations was shown. In the control group of infected rats which do not received any drug treatments, the complete wound healing activity was observed in 12 to 14 days. While in the paraffin alone treated group, the healing activity was observed in ten days. Both *Piper betle* and *Nyctanthes arbortristis* also showed the complete healing in 10 days.

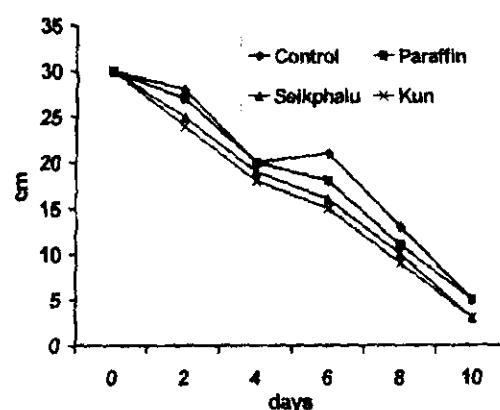


Fig.1. Effect of *Nyctanthes arbortristis* (Seikphalu) and *piper betle* (Kun) on the open wound preparations

The difference between the tests and paraffin group revealed that in the paraffin group there was a formation of pus and induration up to five days while in the tested groups no formation of pus was obtained. In the control group without any treatment the

formation of pus was obtained up to the sixth day.

Effect of Nyctanthes arbortristis and Piper betle on the tensile strength of open wound preparation after post-operative period of 14 days

The effect of *N. arbortristis* (1% ointment in paraffin) on the tensile strength in rats show $195.5 \pm \text{SD} = 17.8$ grams. Similarly the effect of *P. betle* (1% in paraffin) in rats after 14 days reveal $194 \pm \text{SD} = 15.8$ grams. In a group of control without no treatment, the tensile strength obtained as $182 \pm \text{SD} = 15.9$ grams. It has shown that the treated groups possess more tensile strength than the group receiving no treatment (Fig. 2).

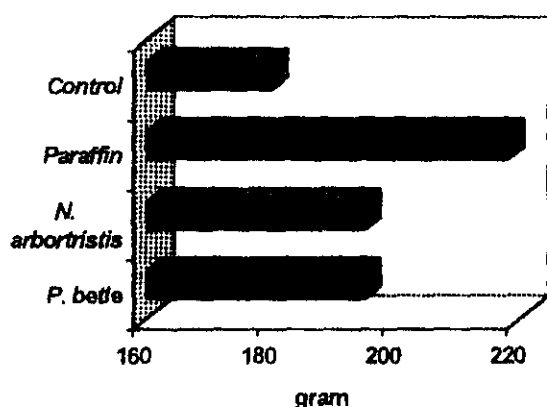


Fig.2. Effect of ethnolic extract of *Nyctanthes arbortristis* (Seikphalu) and *piper betle* (Kun) on the tensile strength of open wound preparation after post-operative period of 14 days.

DISCUSSION

The search for antibacterial properties is worldwide. The constituents of plant compounds and their structural elucidation were also illustrated in this modern era [11-16]. In this research, it is to collect some biological data from the plants in Myanmar which are used for medicinal purposes. *In vitro* study reveals that *Ageratum conyzoides*, *Coleus aromaticus*, *Cuminum cyminum*, *Foeniculum vulgare*, *Nyctanthes arbor-*

tristis, *Phallanthus emblica*, *Piper betle*, *Piper nigrum*, *Terminalia chebula* and *Vinea rosea* showed antibacterial activity regardless of the fact that they possessed variable zone sizes.

Control of methicillin resistant *Staphylococcus aureus* in hospitals is now particularly important in wound infections [17,18]. Moreover, the spread and carriage of *Staphylococcus aureus* is far more important in the population. Thus, if a ready made balms could introduce for local use burns it might decrease the spread of infection and inhibit to produce into large infected wounds. *Piper betle* accelerated the rate of wound infections in infected rats when compared with the controls in *in-vivo* tests. In the control group (without any drug) the complete healing activity was found within 12 to 14 days and paraffin alone treated group it was observed in 10 days. *Piper betle* also showed the complete healing rate within 10 days. The difference between the test and controls revealed that in the control group (without any drug), the formation of pus and induration occurred up to sixth day. Even in the paraffin alone treated group pus formation occurred up to fifth day. Again, *Nyctanthes arbortristis* and *Piper betle* extracts when applied topically gain the tensile strength in wound preparation. Thus, these two plants might be useful in wounds and sores as an emergency use locally. The application of topical use by modified method might be useful to prevent sores and burns to be infected.

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Leprosy related knowledge of community members: dissemination made through MCWA communicators in Bago (West) Division

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Post-intervention evaluation study was conducted in Bago (West) Division. Multistage sampling was used. A total of 1510 females 18 years and above was selected. Out of 1104 respondents (73.1%) who said that they knew the early sign of leprosy, 956 (63.3%) could correctly answer the appearance of patch over the body. Among all, 529 (35%) knew the disease was caused by an infectious agent. They obtained leprosy related knowledge through health staff rather than MCWA communicators. Regarding the knowledge scores, 695 (63%) got high scores (10-14), whereas 166 (15%) had low scores (0-4). The difference of knowledge scores was not statistically significant according to residence, occupational status, and MCWA membership. However, the difference was found in 3 townships according to age groups, marital status, and years of schooling. The current dissemination channels using MCWA communicators need further reinforcement.

INTRODUCTION

Leprosy is a chronic communicable disease and also a public health concern because of the permanent progressive disabilities and its social consequences. The global prevalence rate of registered cases stood at 1.25 per 10,000 in January 2000 [1]. The elimination target set at less than one registered leprosy case per 10,000 population by mainly based on improving community access to diagnosis and widespread implementation of multi-drug therapy (MDT) [2].

Myanmar is one of the four countries in South East Asia region, where special efforts are being intensively implemented for the elimination strategy. The prevalence rate was 5.9 per 10,000 at the start of 2000. There was a significant increase of New

Case Detection Rate (NCDR) to 61.8/100,000 (1999) from 19.7/100,000 (1994) [3].

The training of Myanmar Child Welfare Association (MCWA) members on dissemination of leprosy related knowledge among females 18 years and above was done in six hyper-endemic divisions including Bago division to explore hidden cases for the achievement of early diagnosis and treatment [4]. It took place in three phases: at the district level, at the township level and at local levels down to villages [5].

Mothers, teachers, members of local social organizations, respected persons and socially active persons were designated as priority categories for dissemination of message. The key message to be disseminated were:

- the early sign of leprosy is a patch on the skin, which may be hypopigmented or reddish or copper colored, and which is not itchy, painless and numb;
- to go to the nearest health facility in case such a patch is discovered and anti-leprosy drugs are available from any health center.

Evaluation on the effectiveness of the activities of MCWA is needed specifically basing on the achievement as regards the extent to which community members are aware of the key messages being disseminated.

Objectives

The general objective was to explore leprosy related knowledge, disseminated through MCWA communicators, of the females 18 years and above, in Bago (West) division. The specific objectives were to assess leprosy related knowledge of the females 18 years and above, and to identify the source of information for early sign of leprosy among the females 18 years and above.

MATERIALS AND METHODS

A cross-sectional study was carried out among females 18 years and above residing in Pyay, Gyobingauk and Tharyarwaddy townships. A total of 1510 females of 18 years and above were involved in the study. There were 500 samples each in Pyay and Gyobingauk townships and 510 in Tharyarwaddy Township. In each township, 150 samples were chosen from urban areas and 350 samples were chosen from rural areas.

At the township headquarter, two residential areas of MCWA communicators were selected randomly. 75 households were then randomly chosen from each ward.

For rural area, one of the RHCs was chosen randomly. The village tract, residence of at least 5 MCWA communicators, were

included. From the selected villages, a total of 175 households were chosen at random.

Another village-tract, among 3-5 village tracts in the RHC jurisdiction and which is located at least 3 miles away from the SH/RHC village, was chosen randomly and 175 females were selected as in the previous village tract. The study period covered from May to August, 2001. Face-to-face interview, using the structured cum semi-structured interview questionnaire, was taken after pretest. Consent form was also attached.

Data analysis was performed using EPI-INFO software. Knowledge scores were categorized as low (0-4), medium (5-9) and high (10-14), basing on the correct answer of patch as an early sign of leprosy, the features of the patch, cause and transmission of leprosy, and the place where anti-leprosy drugs were available. Scores were cross-tabulated with socio-demographic characteristics of the interviewees.

RESULTS

Mean age of the respondents was 37.8 ± 13.1 and the eldest was 79 years. Mean years of schooling was 5.7 ± 3.2 . Most of them 76.2% were married and 76.6% were multips. Half of the respondents were self-employees and government employees constitute only few percentages. Out of the total respondents, only 4.4% were MCWA members.

Although 1104 respondents (73.1%) said that they knew the early sign of leprosy, only 956 (63.3%) could correctly answer the appearance of patch over the body. Of 956, majority (60.1%) responded the patch was hypo-pigmented, whereas 19.6% knew that the patch might be copper colored also. More than half of them replied that it was painless, numb, and not itchy. One third of the interviewees knew the cause of leprosy by an infectious agent. More than 90% indicated the health center or township

hospital as the place where anti-leprosy drugs were available. Few percentages believed that these drugs were only available from leprosy (Table 1).

Table 1. The interviewees correctly responding the questions related to the key messages for leprosy

Knowledge on leprosy	n	Frequency	%
The respondents expressing the early sign of leprosy: a patch	1510		
• Responding YES to the question: Do you know the early sign of leprosy?		1104	73.1
• Could correctly mention appearance of patch over the body.		956	63.3
In-depth knowledge of the interviewees who could correctly answer the "patch"	956		
• *Color of the patch			
- hypopigmented		575	60.1
- red		572	59.8
- copper		187	19.6
• Sensation of pain		808	84.5
• Sensation of heat		718	75.1
• Sensation of touch		802	83.9
Sensation of itchiness of the patch		577	60.4
The respondents answering correctly the cause and transmission of leprosy:	1510		
• Leprosy is caused by an infectious agent.		529	35.0
Responding YES to the question: Is there person to person transmission of leprosy?		986	65.3
The respondents correctly replying the drugs for leprosy:	1510	1099	72.8
• Responding YES to the question: Are there any curable drugs for leprosy?	1099	997	90.7
If responding YES: Where are these drugs available?			
• There was more than one response for some interviewees.			

The interviewees who could correctly answer the early sign of leprosy had been exposed to health information provided by health staff rather than MCWA members

(20.2% vs. 8.5%). Majority of them (33%) got the knowledge by other channels like videos, television, radio, posters, pamphlets, and newspapers.

The difference of knowledge scores was not statistically significant according to residence, occupational status, and MCWA membership ($p>0.00$). However, the statistical significance was found in 3 townships according to age groups, marital status, and years of schooling ($p=0.00$).

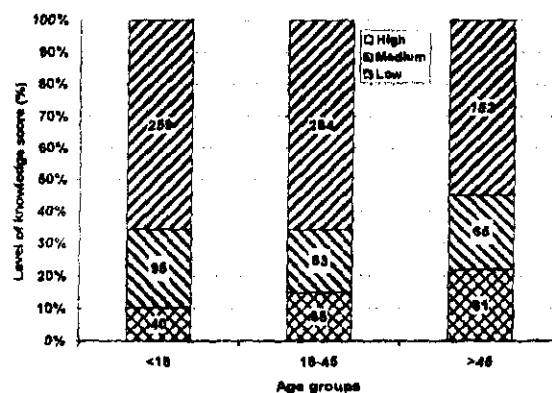


Fig.1. Level of knowledge scores by age groups

Figure 1 highlighted that 695 respondents obtained high scores and 166 respondents had low scores (63% vs. 15%). More than half of each age group gained the high scores. Around 20% of each age group had medium score. In comparison of low scores, the highest percentage was seen in over 45-year age group whereas the lowest percentage was observed in 18-30 year age group (22% vs. 10%).

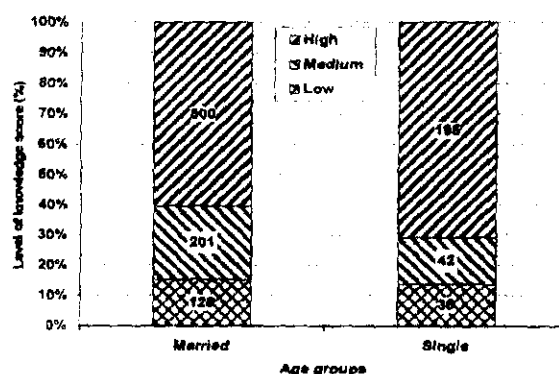


Fig.2. Level of knowledge scores by marital status ($p=0.00$)

Scores were categorized according to marital status in Figure 2. Majority of the respondents were married. The singles achieved high scores rather than the married (70.9% vs. 60.3%). However, the percent-ages of medium and low scores by the single were less than that of the married (15.3% vs. 24.2%) and (13.8% vs. 15.4%).

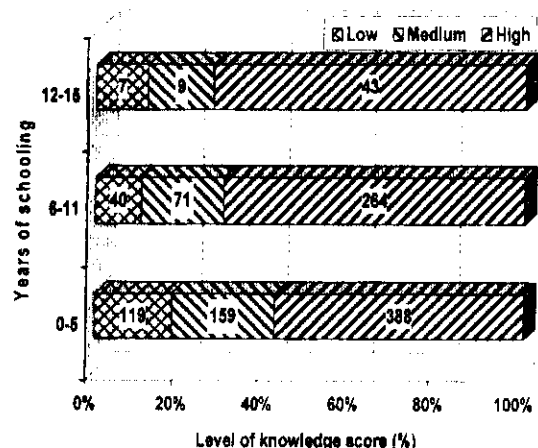


Fig.3. Level of knowledge scores by years of schooling ($p=0.00$)

Figure 3 represented the level of knowledge scores by years of schooling. It was found that 12 to 15, 6 to 11, and 0 to 5 years of schooling group were high score winners in order of frequency, vice versa for medium scores. For low scores, the highest percentage was found at 0-5 years of schooling compared to 6 to 11 years of schooling, which constituted the lowest percentage.

DISCUSSION

From the above findings, not all the respondents who said they knew the early sign of leprosy could mention appearance of patch over the body. The early sign could not be identified. Very few could indicate the exact color of the patch. Provision of MDT drugs by health center should be explained to the public because majority thought that MDT drugs were given by health staff who are only assigned for leprosy. The key points like early sign,

cause, and transmission of leprosy should be highlighted both in IEC materials and health education programme used for community. From the study, the respondents were found to be more exposed to leprosy related health information through health staff rather than MCWA. Hence, the current dissemination channels using MCWA communicators need further reinforcement.

Although majority of the participants was married, the singles seemed to gain higher scores than the married. It should be taken into consideration that the community members who should be reinforced in health education process were those who had the highest percentage of low scores: over 45-year age group, the mothers, and those with low educational level. The results would be useful in launching an appropriate programme for promotion of dissemination on leprosy control through NGOs.

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Perception towards self-care among older women in rural area in Taik-Kyi Township, Myanmar

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This study aims to assess the attitude and practices related to self-care among older women in rural areas. It was conducted in the village tracts under Taw La Ti station hospital area, Taik-Kyi Township, Myanmar in October 1999. Techniques of Participatory Rural Appraisal (PRA) were used for data collection. The qualitative methods: In-Depth Interviews (IDI) were conducted among 19 older women with different background characteristics regarding age, marital status and type of family. Ages of the respondents ranged from 63 years to 90 years. Majority of the older women interviewed could do their personal activities and household chores. Although the respondents define good health in different ways, the basic concept was related to physical activity. All older women in the study mentioned self-care as prevention of accidents and fall. Regular medical check-up was not a usual practice among older women. Regarding health needs, almost all of the respondents stated that they were looked after and supported by their family and the community. However, they said that no specific health care services for older person existed in their localities. Almost all of the grandmas in this study played active roles not only in their families but also in the community.

INTRODUCTION

One of the major achievements of the 20th century has been an increase in life expectancy in almost every country in the world. At the same time, fewer children are being born as people become more able to plan their families. These two factors mean that across the world populations are getting older -with a high number of older people and a smaller proportion of younger people. This population change is happening fastest in less-developed countries. By the end of 1995, the number of people aged 60 years and over across the world increased by more than 12 million (over one million people a month). Nearly 80% of this increase took

place in the developing world [1].

Older people in Myanmar (60 years and above) are about 2.6 million in 1990-1991 which is 6.4% of total population and expected to increase further in future [2]. In Myanmar, the aged dependency ratio is 11 in 1990. It is expected to increase to 13 in the year 2020 [3]. Female population over 65 years of age in relation to male population over 65 years of age stood at 1.2 in Myanmar [4].

Aging is not a disease but a normal part of the life process. The older people face a number of social and personal problems such as high rates of physical illness and

emotional difficulties, malnutrition, lack of meaningful role in society. Aging is inevitable and irreversible but does not automatically lead to ill health. Many of the health conditions associated with aging can be prevented or delayed if people were accessed to decent living conditions and suitable health care throughout their lives. If older people receive health education and medical attention whenever it is necessary, they are less likely to develop serious medical conditions and complications which are more difficult and expensive to treat [4].

Nearly everywhere, women live longer than men. So, the older world is also a female world. Globally, just over half of the older population aged 60-69 years are women and 65% of those aged 80 years and over are also women. Because of demographic, cultural and income differences between genders, aging means more challenges for women than for men.

Therefore, there is a great need to identify health needs and knowledge, attitude and practices of self-care among older women in our country. Self-care has been defined as a process in which people function on their own in health promotion and prevention, and in disease detection and treatment at the level of the primary health resource in the health care system [5]. The health sector alone cannot ensure adequate comprehensive support services for them. It can only be fulfilled through the concerted efforts of all government departments, social support groups, religious organizations and active community participation.

The National Health Plan 1993-1996 and 1996-2001 of Myanmar includes substantial sections on health care of the elderly. According to WHO Regional Strategy for Health Care of the Elderly (1992-2001), all regional countries will have to undertake measures to create mass-awareness regarding the needs of the elderly [6]. Thus, by understanding the perceptions towards self-care of the older women, it is believed that

health care for the older women will be improved much better. Besides, it will also enhance the health education program and a useful tool for health planning for older women. This study may also make awareness of the community to the problems and issues of older women.

Objectives

General objective

To assess the knowledge, attitudes and practices related to self-care among older women in rural areas in Taik-Kyi Township.

Specific objectives

1. To determine the knowledge and attitudes of older women in rural areas in Taik-Kyi Township about self-care.
2. To find out the treatment-seeking behavior of older women in rural areas in Taik-Kyi Township.
3. To assess the perceived health needs of older women in rural areas in Taik-Kyi Township.

MATERIALS AND METHODS

Design and Methods

Study design

It is a qualitative study using Participatory Rural Appraisal (PRA) technique. PRA tools that used in the study included:

Preparation and training of team members

Three teams for data collection in the field were formed. There were 2-3 team members in each team. The team members consisted of one research officer and 1-2 research assistants. All team leaders and members possess field experiences in qualitative data collection methods.

Building local teams within each village in the study area

Local teams were formed with older women in the study area. The action plan of each

village was guided by the local teams.

Observation

Direct observation of activities of older women was done in the community.

Conducting in-depth interviews (IDI)

IDI in the form of interviewing was conducted among 19 eligible older women. The eligible respondents were chosen by local team members according to their different background characteristics (age, marital status and type of family).

Study area and study population

Study area was the village tracts under Taw La Ti station hospital, in Taik-Kyi Township, Myanmar. Study population was all older women of aged 60 years and above residing in the study area.

Data collection methods

Participatory Rural Appraisal technique was used for data collection. The 19 eligible respondents were chosen by local team members according to their different background characteristics (age, marital status and type of family). Each in-depth interview for each client was conducted by the trained moderator with assistance of a note taker and a cassette recorder.

Data analysis

The discussions were transcribed, translated and thematically coded. Commonalties were extracted through words, phases and themes and re-coded and summarized. Then the analysis framework was developed. Matrix analysis was performed according to main themes and sub-themes.

RESULTS

1. Background variable

All of the respondents were the residents of the village tracts in Taw-La-Ti station hospital area in Taik-Kyi Township. Ages of the respondents ranged from 63 years to

90 years. Among 19 respondents, 7 were widows and 2 were singles. Majority lived with their family apart from 3 older women who lived alone. A few of the respondents had their own earning. Most of the older women were educated up to primary level and only a small number were uneducated.

2. Daily Activities

Daily chores were usually carried out by majority of the respondents. Their personal activities such as changing clothes, having baths, eating and walking were done on their own. Being Buddhists, all of the older women spent most of their time on religious works such as praying, offering alms and meditation. It was very pleasant to hear from the elders that the first and foremost activity done for the day was praying and worshipping Buddha.

Majority of the interviewees enjoyed cooking for the family except 2 very old grandmothers. The reason for that was family members were worried about their health and prevented them from working. Other household activities done by the elderly were taking care of grandchildren, cleaning the house and its environment. Most of the respondents enjoyed doing household chores because they were used to it.

"I got up early in the morning, did all the cooking, preparing lunch boxes for my grand children and taking care of the small one in the afternoon. I occupied myself like this everyday". (65 years old widow living with family. She stated this issue with a peaceful smile in her face)

However, another older woman said:
"I am fed up with cooking, fetching water and all sort of things but I have to do such things because I have no choice" (65 years widow living with family)

3. Health

3.1. Regarding question on "what do you understand by good health"

Although the respondents defined good health in different ways, the basic concept was related to physical activities. Only a few mentioned about mental well being.

"Health is nothing but just an ability to do routine activities like eating, sleeping and walking" (82 years old widow living with family)

"Health is a state in which having not only good physical activity but also enjoyable time with family and no socio-economic burden. A peace in mind is also essential" (77 years old widow living with family)

3.2. Perceptions of Health status and health problems.

Regarding the health status, half of the interviewees thought that they were quite healthy. However, the rest of the older women stated that they were not healthy because they had some ailments such as dizziness, tiredness, loss of appetite, etc.

90 years old grandma who lived with her family said *"I can walk around. So, I feel healthy. I'm not bed ridden yet"*.

"I'm very unhealthy unlike my husband who is over 90 years old. I'm suffering from shortness of breath (difficulty in breathing), tiredness and all sorts of things. So I could not go to the monastery today - full moon day of Thidingyut. I feel so sorry for that. What can you do for me ?" (88 years older woman living with spouse only)

Only a few of the older women interviewed could identify some health problems. They were not quite aware of their health problems. Common health problems spelled out by a few respondents were joint pain, *"thwe-toe"* (high blood pressure), stroke, hearing problem, eye problem, loss of memory and general weakness.

4. Self - Care

4.1. Prevention of accident and fall

All the interviewees were quite aware that

they were prone to get accident and fall. Therefore, they all took care of themselves in various ways to prevent fall.

"I always take care of myself to prevent fall and injury. I have 4-5 walking sticks in-hand". (78 years old single who was living alone)

4.2. Diet pattern (eating behavior)

Most of the respondents have no special avoidance of food. However, there was avoidance of certain food in about one third of the interviewees. The common foods that they avoided were salty food and foods that might cause wind colic. On the other hand, there were some respondents who took special food for their health and fitness.

Eighty-eight years old widow who lived with family said : *"I have no special food avoidance because I feel healthy and happy only when I eat whatever I like"*.

"I avoid taking food which may cause hypertension and indigestion such as salty diet and pork" (99 years old widow living with family)

One of the interviewees stated *"I always take citrus fruits with sugar to relieve abdominal fullness. I also took jaggery for digestion"*. Then she continued with a smile when you grow old, you'll know better. There is a saying that *Ayware-O-ah-cho-kyike* (when getting older, preferring sweet diet)". (88 years old married woman living with spouse only)

4.3. Regular taking of medicine and drugs

Almost all the older women in the study took one kind or other forms of folk medicine regularly for their health and fitness. However, a few older women did not have either indigenous medicine or western drug in-hand.

"I take 'Thwe-Say' with sugar to get good digestion. It was advised by other people of

our age. I also take some vitamins because my son bought them from Yangon ". (76 years old woman living with spouse only)

"I did not have any drugs or medicine in-hand. Because it was not necessary for me. I bought and took drugs from local shop only when I felt unhealthy ". (65 years old widow living with family)

4.4. Regular taking of exercise

Routine physical activities were regarded as a kind of physical exercise by majority of the respondents.

"I have done physical activities by taking care of my grand child ". (90 years old widow living with family)

"Early in the morning, I did my cooking, cleaned the house and sold fishes in the village. Such activities make me healthy ". (65 years old widow living with family)

4.5. Medical check up

Regular medical check up was not a usual practice among the interviewees. Although most of them thought it was not necessary to take regular check up, only a few would like to consult with health personal regularly and had taken regular check up.

"I've never done medical check up because I think it is not necessary and I'm quite all right. Isn't it?" (77 years old widow living with family)

"Although I would like to consult with someone, I mean health personal, there is no 'saya' (practitioner) here. You see! So, we couldn't do anything ". (88 years old woman living with spouse only)

5. Treatment seeking

5.1. Initial management

The commonest symptoms that most of the older women interviewed first noticed were loss of appetite, dizziness and feeling of

weakness. Almost all of the married older women usually shared their feeling of sickness with their off springs especially daughters. Older women who lived only with their spouses usually did not tell about their sickness to anyone. 88 year old women who lived with her spouse only stated that she did not tell anybody when she was sick because she was reluctant to give burden to others. She only let her husband and neighbors know about illness only when she could not withstand the condition.

"My daughter was the first person who noticed my illness because I have one and only daughter and she was so intimate with me". (77 years old widow living with family)

Most of the women interviewed practiced self-medication by taking either indigenous medicine or western drugs.

"My daughter put some traditional medicine in the bottle and labeled them. I took these drugs when I didn't feel well ". (90 years old widow living with family)

"I took neurobion to relieve giddiness. My friends gave advice to do so ". (78 years married older woman living with family)

5.2. Home management/care

Most of the respondents mentioned about special diet during their illness. They took special diet (*Dut- Sar*) to get better. According to their sayings, there were various kinds of food, which were recognized as '*Dut-Sar*' such as citrus juice, cow's milk, and honey. Some respondents stated that they took massage by a masseur or a relative to relieve joint pain and stiffness. Major care-givers for the grandees were their daughters and their grand daughters.

"Actually my son did not know how to take care of me when I am sick. My daughter-in-law was not really a relative by blood you know! So, my daughter came and took care of me when I was sick. Then I got well within short period ". (78 years married

older woman living with spouse only)

"My daughter was the one who usually takes care of me when I'm sick. She not only gives me the appropriate drug but also prepares diet for me. My youngest son went to consult with 'saya' in other village ". (77 years old widow living with family)

5.3. Utilization of health service

Almost all of the interviewees usually contacted health personnel only when they were not relieved with home remedies within 2-3 days. Most of the respondents stated that they usually followed the instructions given by the health personnel. The instructions mostly given were to take drugs regularly and to come again if the illness was not relieved.

"When I was sick, the sickness usually relieved within few days with folk medicine. So, it was not necessary to go to clinic ". (76 years old widow living alone)

6. Perceived health needs

Regarding health needs, all respondents mentioned that there were no health care services for older people in their locality. They gave different kinds of opinion for better health of older women. All of them requested to provide health service by qualified health persons in their villages and regular visiting of health care team to their villages. They also mentioned about transportation problems and lack of attendants to go to health center in other villages. Some respondents also gave suggestion to offer foods and clothing to older people who were in need.

"To have a clinic, I mean a real clinic opened by a trained health personal, is good or regular visit of health care team is not so bad. Say-Hto-Saya (Injectionists) here are not trained persons and they even don't know how to do it. Please come and open clinic here. We shall provide lodging, food and everything for you. No need to worry

about". (88 years old woman living with spouse only)

7. Support for older women

7.1. Family support

Almost all of the married older women were willing to tell about the family support they received. But those older women who lived alone or lived with spouse only were lacking family support. However, most of these respondents mentioned that neighbours and other relatives who lived close to them gave certain kinds of support such as taking them to clinic or bringing food and drugs for them when they were sick.

"My daughter and daughter-in-law take care of me. They do all washing and fetching water for me. They even did sponging when I was ill". (77 years old widow living with family)

7.2. Community support

More than half of the respondents stated that they received community support.

"People in the village also support older persons by giving medicine and food (Dut-Sar). I have lived up to this age by all their support ". (80 years old woman living with spouse and family)

"People in the village came to me and asked' what can we do for you? 'when I was ill. They took ' saya ' in case of emergency at night and they did cooking and other housework if I was bed-ridden. This is a lovely "da lae"(habit) of village". (65 years old widow living with family)

8. Role of older women in society

Most of the respondents were willing to tell their active role in the family and community. Almost all of the older women who lived with their family stated that they were regarded as decision-makers. They had decision-making role in marriage affairs of

their offsprings. Moreover, they also managed all family affairs such as giving advice for social and economic affairs of household. Most of the interviewees also took part in the social occasions of the village such as marriage ceremonies. They managed not only all the cooking but also dating the occasion.

"As we grow older, we have more experience on life so we could give certain advice" (81 years old woman living with spouse and family)

9. Outlook on life

Older women interviewed had different views of outlook on their life. Some had better outlook than others. Majority of the older women who lived with their family had positive thinking on their life. However, some older women who lived alone or lived only with spouse had unsatisfactory thinking of their life.

"Life is usually a mixture of happiness and sorrow. So I don't want to make any comment on my life. I only spend most of my time on religious work which keeps me peaceful" (76 years old woman living with spouse only)

"I was happy and pleased when I was young. But now I feel unhappy and sometimes I am not satisfied because I have no companion and no attachment. Moreover, I have some economic problems in my old age". (66 years old single woman living alone)

DISCUSSION AND CONCLUSION

In this study, majority of the older women interviewed not only could do their personal activities but also enjoyed doing household chores as a routine. It was found that they were the major caretakers for their family especially at home. Their works were important contribution to family life. These works enabled other family members to seek paid work outside home and earned an

income for the family. Thus, they should be regarded as "hidden workers" for the society.

Regarding good health, the respondents defined it differently in their own ways. Physical well being was the major emphasis mentioned in their definition of good health. Only a few mentioned about mental well being. They all thought that physical activities were declining, as they get older. There is a Myanmar saying "*Seik Thwa Taing Ko Ma Pa*" which means physical performance cannot follow the mind as it used to be in the younger days. Therefore, an older person's felt need about good health should be taken into consideration in the care of the elderly. In this study, it was evident that most of the older women regard themselves to be healthy and free from health problems. The common health problems as spelled out were joint pain, *thwe-toe* (high blood pressure), general weakness and stroke. During their illness, majority of the respondents followed the advice given by their neighbours and friends for initial treatment at home. Moreover, daughters were mentioned as the intimate care-givers among the family when older women were sick. This pointed out that neighbours and family members were the priority groups to give health education on care of the elderly.

In this study, many older women were cared for at home by family members, relatives, neighbors and friends; but they received very little support from social and health services. Thus it highlights that there should be health care providers trained to help elderly in the rural areas. There are many informal voluntary services in rural areas for the elderly. Therefore health care providers should be hand in hand with informal volunteers in the care of the elderly.

On the topic of self-care the older women in the study expressed it as prevention of accidents and falls. To keep them healthy and active they usually take a special diet

called "Dut-Sar" and avoid certain foods like too rich, too oily, too salty foods which may cause them ill health. In this study, most of the older women took one kind or other forms of indigenous medicine on regular basis. Medical check-up was not a usual practice among the study group. They perceived the medical check-up as an unnecessary procedure for them. Only a few would like to consult a health personal for their health regularly. Along with the belief in traditional medicine, they also have a positive attitude towards health care services in their word "Ah Naught Taing Say"(western medicine).

Almost all the grandmas in the study played an active role in their families as well as in the community. There is always a traditional empowerment of older people to pass their knowledge and skill to the younger generation for a brighter future. Myanmar people also believe that older people are the guardians of local history, tradition and culture. Therefore, these findings are also the positive aspects for empowerment of older women in the society. Moreover, it will help in the future care of the elder people, which we preserve as our nation's treasure.

Recommendations

1. Appropriate health care services involving volunteers should be established at local level for the elderly.
2. Health education on the importance of elderly care should be given to the immediate family members as well as younger generations.
3. Strategies that will enhance the involvement of older people both as

providers and receivers in the community should be strengthened.

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**Effect of heat inactivation on Hepatitis B surface antigen prepared by
The New York Blood Center method**

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Inactivation phase is necessary to eliminate any residual microbial particles in the process of hepatitis B vaccine production. The present study is aimed to assess the effects of heat inactivation procedure on purified hepatitis B surface antigen (HBsAg) obtained by New York Blood Center (NYBC) method. Electron-microscopic (EM) examination and polyacrylamide gel electrophoresis (SDS-PAGE) were carried out on purified HBsAg preparation before and after heat inactivation. Clumpings of HBsAg particles were observed under the EM after heat inactivation, which rendered the antigen more immunogenic. After the double step heat inactivation, no influence of the inactivation process was recognized on the SDS-PAGE results. The plasma-derived hepatitis B vaccine met all the quality control tests results including General Safety test recommended by the World Health Organization.

INTRODUCTION

Currently licensed plasma-derived hepatitis B vaccine consists of purified 22 nm HBsAg particles extracted from the plasma of chronic carriers [1]. Source plasma could contain infectious agents that possess various degree of susceptibility or resistance to different modes of inactivation. Thus, procedures which will inactivate all infectious agents present in human blood should be applied during the vaccine manufacturing process. Several methods have been used and approved by WHO for the consistent production of safe batches of vaccine which include chemical treatment, physical or heat inactivation consisting of flash heat treatment (101-104°C for 90-160 seconds) followed by pasteurization at 60-65°C for 10 hours [2,3].

A simple inactivation method devised by Prince *et. al.* [4] consists of heating at 102°C for 2 minutes 40 seconds followed by pasteurization (65°C, 10 hours). This heat inactivation procedure has been applied on HBsAg purified by the NYBC method [4]

and its effect has been investigated in the present study.

MATERIALS AND METHODS

Source of Starting Material

Plasma containing high titre of hepatitis B surface antigen (HBsAg) was obtained from the National Blood Bank at Yangon General Hospital and other hospitals within Yangon area.

Purification

The plasma pool was subjected to three successive steps of differential precipitation with polyethylene glycol. Then the antigen was further purified by batchwise adsorption on hydroxylapatite and selective elution of HBsAg to remove soluble serum proteins especially albumin. Then the antigen solution was concentrated by ultrafiltration to reduce the final volume. The HBsAg was then purified by two successive ultracentrifugations using linear density gradient of KBr (density ranging

between 1.05 gm/mL and 1.35 gm/mL), at 29,000 rpm for 19 hours. Then, the fractions containing HBsAg were collected, and dialysed against saline to remove KBr.

HBsAg purity

HBsAg purity was checked by electron-microscopy and sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Inactivation procedure

Aliquots of the purified antigen were adjusted by using pyrogen-free saline to get concentration of 0.5 mg protein/ml and filtered through a 0.22µm millipore filter.

The antigen preparation was passed under pressure through a stainless steel coil held at 102°C in flash heating equipment (oil bath) at a rate such that the material will be held at that temperature for 2 minutes 40 seconds. And then the inactivated antigen was pasteurized at 65°C for 10 hours as an additional inactivation step.

The heat inactivated antigen was then tested for the followings:

- (1) Protein concentration was measured by the method of Lowry *et al.* [5]
- (2) Electronmicroscopy examination
- (3) SDS-PAGE by Laemmli. Molecular weights were determined by comparing with standard Low Molecular Weight Calibration Kit [6]
- (4) General Safety Testing was carried out according to the procedure recommended by World Health Organization for Hepatitis B Vaccine [2,3]. Six healthy mice, each weighing 17-22gm were selected, two for each test (Lot 01 & Lot 03) sample and two for test control. The two healthy mice were inoculated intraperitoneally with 1 ml of heat inactivated HBsAg containing 10 µg/ml of protein. Normal saline 1ml was injected into a mice selected for negative control. The mice were

observed and weighed daily for one week and their daily weights and any abnormal response were recorded.

RESULTS

As shown in Fig. 1 microscopic examination revealed that hepatitis B antigen preparation consisted of 22 nm spherical particle and did not contain any whole virus or Dane particle.

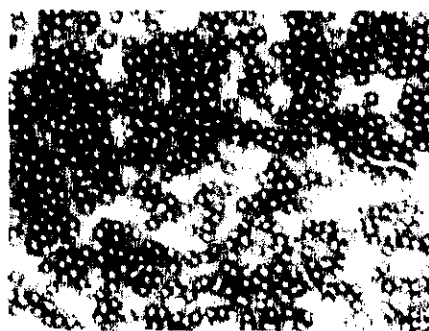


Fig.1. Electron micrograph of purified HBsAg



Fig.2. Electron micrograph of HBsAg
(Post pasteurization after filtration)

Fig. 2 shows that the same spherical particles in the post filtered HBsAg. The HBsAg particles were aggregated after heating at 102°C for 2 minutes 40 seconds as shown in Fig.3.

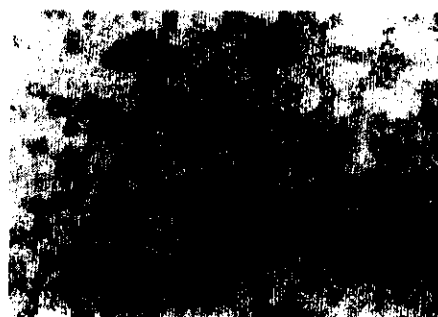


Fig. 3. Electron micrograph of post heat HBsAg

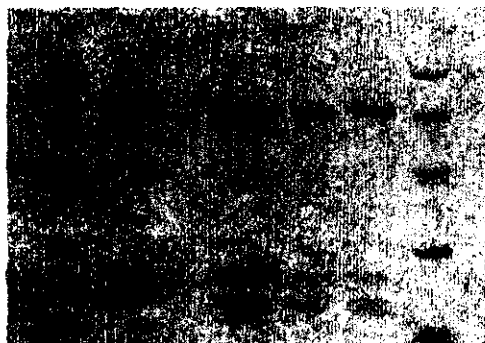


Fig. 4. Polyacrylamide Gel electrophoresis
(a) Low molecular weight markers
(b) 01 post heat
(c) 03 post heat
(d) 01 purified HBsAg
(e) 03 purified HBsAg

Fig. 4 shows an analytical electrophoresis on SDS-PAGE gel of purified HBsAg. Two bands corresponding to molecular weights ranging from 22,700 to 24,400 and 26,600 - 28,500 are significant. They are probably the major polypeptide (P-24) and its glycosylated form (gp 27) present in the 22 nm HBsAg particles. One minor band of molecular weight about 41,310 is also observed and this probably may be the peptide derived from pre S region. One minor band of molecular weight about 69,000 is also observed which may represent the serum albumin.

Table 1. Protein concentration of purified HBsAg before and after heat treatment

HBsAg Lot No.	Protein Concentration mg/ml		
	Pre-filter, Pre-heat	Post-filter	Post-heat
01	0.5	0.400	0.30
03	0.5	0.475	0.33

Table 1 shows the changes of protein concentration during the process. In fact, the slight decrease in protein concentration was showed during the process. In General Safety test, all mice survived during the test period and showed no behaviors changes. The body weight of the test groups showed normal increase in body weight as can be observed by comparing with the control

group, as shown in Table 2. Thus the heat inactivated antigen passed the general safety test.

Table 2. General safety test of purified HBsAg after heat treatment.

Sr. No.	HBsAg Lot No.	Test dose & route	Test animals	Body Weight (gm)	
				Day 1	Day 7
1.	01/93	Intra-peritoneal 1ml each	Mice no. 1	20.0	24.0
2.	01/93	"	Mice no. 2	21.0	23.0
3.	03/93	"	Mice no. 1	20.0	23.5
4.	03/93	"	Mice no. 2	21.0	23.0
5.	Control (saline)	"	Mice no. 1	20.0	21.5
6.	Control (saline)	"	Mice no. 2	20.0	24.0

DISCUSSION

It has been claimed that the flash heating and pasteurization can eliminate any residual viral particles that can be present in the purified HBsAg during plasma-derived hepatitis B vaccine production. HBsAg particles are 22 nm spherical particles which represents the outer coat portion of the hepatitis B virus. Only Dane (42 nm) particles are infectious. Electron microscope examination was done on purified HBsAg before heat inactivation to assure the absence of the infectious hepatitis B virions or Dane particles. It was found that our antigen preparation consisted of only 22 nm empty spherical particles and did not contain any Dane particle. The particles were not effected by the inactivation process except that HBsAg particles were aggregated after heat inactivation which may increase in immuno-genicity of the HB vaccine. In PAGE under denaturing conditions the purified antigens were resolved into several major protein species.

The results of SDS-PAGE are the same before and after heat inactivation as evident in fig 4. The inactivation process did not affect the SDS-PAGE results. This is in accordance with the findings of previously published data (Field, Korea C.J.) [7,8]. Albumin is one of the most frequently encountered components associated with purified HBsAg. Plasma protein components are not considered as contaminants but rather represent integrated proteins within the viral envelope (Vnek *et al*, 1978, Pillot 1979) [9].

In general safety test the results met the requirements as recommended by the World Health Organization [2,3]. All the results were satisfactory to exclude extraneous toxic contaminants.

In conclusion, the inactivation procedure used for the hepatitis B vaccine production as shown above will be useful to carry out further safe and effective vaccine in Myanmar.

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SHORT REPORT

Geographical variation of biological properties of Russell's viper (*Daboia russelii siamensis*) venom: Mandalay Division

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Geographical variation of biological properties of venom of different snakes has been reported [1-4]. Snakebite is endemic in six rice-growing divisions of Myanmar and biological properties of venom from Ayeyarwady, Yangon, Bago and Magway had been studied [5-9]. Understanding of the variation in biological properties and composition of venom to be used as an immunogen for preparation of antivenom plays an important role. This report concerns the study of biological properties of venoms from Mandalay Division.

Snakes collected from three localities of Mandalay Division (Kyaukse, Thazi and Wundwin) were milked individually. Venoms were pooled and lyophilized according to locality and length of the snake into two groups: adult (>80 cm) and young adult (<80 cm). Venom of young

adult snake of Wundwin was not available for study.

Biological properties of venom such as haemorrhagic, necrotic, coagulant, defibrinogenating, lethality and capillary permeability increasing activities were studied according to the WHO recommended techniques [10]. SDS-PAGE electrophoresis of venom was also carried out. Details of the methods have already described [5].

Results of the biological properties of venom from three localities are shown in the table. It was found that the venom of juvenile (<80 cm) snakes of Kyaukse was more potent than that of the adult in lethal and defibrinogenating (2.4 times) and coagulant (30 times) activities, however the latter and weak capillary permeability

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

Source of venom	Length of the snake (cm)	Number of snake pooled	LD ₅₀ iv μg/mouse 1±SD	MCD g	MDD g/mouse	MHD g/rat	MND g/rat	MCPID g/ml
Kyaukse	>80	40	14.35 ± 3.90	0.1355	12	54.33	47.86	>1 mg
Kyaukse	<80	42	6.450 ± 1.88	0.0040	5	52.19	45.19	0.007
Thase	>80	40	3.75 ± 1.94	0.0630	2	54.95	39.31	0.007
Thazi	<80	48	6.306 ± 2.25	0.0200	4	61.66	39.81	0.010
Wundwin	90-102	2	7.25 ± 2.51	0.3162	3	67.61	56.23	0.015

LD₅₀ iv = Median lethal dose

MCD = Minimum coagulant dose

MDD = Minimum defibrinogenating dose

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MCPID = Minimum capillary permeability increasing dose

increasing activity. In contrast, adult venom of Thazi was 2 times more potent in lethal and defibrinogenating activities than the juvenile and the latter was 3 times more potent than the former in coagulant activity. The venom from adult snakes of Thazi was the most potent among three with except coagulant activity.

SDS-PAGE electrophoresis of the venoms shows there were qualitative and quantitative differences in protein bands (figure not shown).

This is the 5th of division-wise study of geographical variation of Russell's viper (*Daboia russelii siamensis*) venom of Myanmar. Observation of intradivisional variation in Russell's viper venom in Mandalay Division supports our previous observation that there is geographical variation in the biological properties of Russell's viper venom in Myanmar [6-9]. It pointed out that the adult venom of Thazi is more potent than other adult venoms. Since the adult venom from Kyauksae possess weak defibrinogenating and capillary permeability increasing activity, if the victim developed signs of increase capillary permeability activity and defibrination, a considerable amount of the venom must have entered into the victim. On comparing with the Russell's viper venoms from Magwe Division [8] the adult venom of Myethe is more potent than the adult Thazi venom in haemorrhagic, necrotic and defibrinogenating activities whereas the latter is more potent in lethal, coagulant and capillary permeability increasing activities. Because of variation in venom properties it is expected that variation in performance of the antivenom and clinical features of the victims will occur. It is note worthy those venoms from juvenile snakes of Thazi and Kyaukse possess potent coagulant activity compared to that of adults.

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SHORT REPORT

Factors influencing approximate LD₅₀ determination of snake venoms using eight to ten experimental animals

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It has been recommended that the number of animals used for laboratory experiments should be kept to minimum and should give minimal suffering as much as possible [1]. Approximate LD₅₀ determination animals [2] could be used in place of clinical LD₅₀ determination of venoms and drugs. However, the values obtained with the approximate LD₅₀ using a random dose range [2] were variable. In order to get an approximate LD₅₀ value closed to classical one, the following experiments were carried out.

A pooled lyophilised Russell's viper (RV) (*Daboia russelii siamensis*) venoms of Lepadon (n=10), Bago division stored at +4°C was dissolved in 0.9% sodium chloride solution immediately before use. Ten male ICR white mice weighing 20g (Laboratory Animal Service, Department of Medical Research) were used for each test. The method of Meier and Theakston [2] was followed. Briefly, venom solution (10 ml/kg) was injected intravenously into the tail vein. Survival times (time between injection and death) were recorded to the nearest 5 sec. Approximate LD₅₀ of the venom was calculated by applying the calculation scheme [2].

For initial dose finding experiment, 1 to 5.5 mg/kg dose range with a dose increment factor of 0.5 was used (Table 1). Based on this initial dose, a series of experiments

were carried out with a dose increment factor of 0.5, 0.25 and 0.125 using ten mice (Table 2). The effect of using 2 mice per dose at five lower doses on the approximate LD₅₀ i.v. values were also tested (Table 3).

Table 1. Initial dose finding experiment of approx. LD₅₀ of Russell's viper venom of Lepadon using a different dose increment

Dose range (mg/kg mouse)	Factor	LD ₅₀ i.v. µg/mouse (µg/mouse)	Classical LD ₅₀ i.v. (µg/mouse) (95% confidence limit)
1-5.5	0.5	45.80	3.29 (3.24-3.34)
0.125-4	0.5	19.19	
0.125-2.37	0.25	10.89	
0.125-1.25	0.125	3.76	

Classical LD₅₀ i.v. of the venom was determined by injection of 0.2 ml of venom in physiological saline into the tail vein of 18-20 g male ICR strain mice. Five mice were used at each venom dose. The LD₅₀ was calculated by probit analysis of deaths occurring within 24 h of venom injection [3].

The classical LD₅₀ i.v. of the venom was 3.29 µg/mouse (3.24-3.34 µg/mouse) (95% confidence limits) and that of the approximate LD₅₀ i.v. (one mouse per dose and two mice per dose) were 3.76 µg/mouse

Table 2. Approx. LD₅₀ experiments of the venom carried out with a different dose increment based on a minimal dose, which kills the animal

No.	Dose (D) (mg/kg)	Survival time (min) (T)	D/T	Animals used for different LD ₅₀ estimation				
				A	B	C	D	E
1	0.125	2.583	0.0483	X	X	X	X	X
2	0.25	2.92	0.0856	X	X	X	X	X
3	0.375	2.83	0.1325	X	X	X	X	X
4	0.5	4.33	0.1154	X	X	X	X	X
5	0.625	2.33	0.2682	X	X	X	X	X
6	0.75	4.75	0.1578	X	X	X	X	X
7	0.875	2.083	0.42	X	X	X	X	
8	1.0	2.083	0.48	X	X	X		
9	1.125	2	0.5625	X	X			
10	1.25	2.83	0.4416	X				

Classical LD₅₀ : 3.29 mg/mouse (3.24-3.34)

Approximate LD₅₀. Calculation based on animal numbers (A-E) according to the calculation scheme

A (n=10) : 3.76 µg/mouse (2.31-5.2)

B (n=9) : 4.07 µg/mouse (2.65-5.49)

C (n=9) : 3.91 µg/mouse (2.27-5.55)

D (n=7) : 3.748 µg/mouse (1.81-5.678)

E (n=6) : 2.275 µg/mouse (± 3.028)

Table 3. Approx. LD₅₀ experiments of the venom carried out with 2 mice per dose at five lower dose ranges

No.	Dose (D) (mg/kg)	Survival time (min) (T)	D/T	Animals used for different LD ₅₀ estimation					
				A	B	C	D	E	F
1	0.125	2.417	0.0517	X	X	X	X	X	X
2	0.125	3	0.0417	X	X	X	X	X	
3	0.25	2.583	0.0967	X	X	X	X	X	X
4	0.25	3.367	0.0742	X	X	X	X	X	
5	0.375	1.883	0.204	X	X	X	X	X	X
6	0.375	2.2	0.1704	X	X	X	X	X	
7	0.5	3.45	0.145	X	X	X	X		X
8	0.5	3.267	0.153	X	X	X			
9	1.625	4.17	0.1499	X	X				X
10	0.625	2.5	0.25	X					

Classical LD₅₀ : 3.29 µg/mouse (3.24-3.34)

Approximate LD₅₀. Calculation based on animal numbers (A-E) according to the calculation scheme

A (n=10) : 3.602 µg/mouse (3.022-4.182)

B (n=9) : 3.264 µg/mouse (1.47-5.058)

C (n=9) : 3.48 µg/mouse (2.059-4.901)

D (n=7) : 3.992 µg/mouse (2.896-5.018)

E (n=6) : 3.642 µg/mouse (2.167-5.117)

F (n=5) : 6.238 µg/mouse (4.375-8.098)

(2.31-5.2 µg/ mouse) and 3.602 µg/mouse (3.02-4.18 µg/ mouse) respectively (Table 2 and 3).

Since 15 times higher LD₅₀ was obtained if

a random dose was used, it is suggested that a minimal dose increment based on a minimal dose, which kills the test animals, should be used. Either 2 mice per dose or one mouse per dose could be used for LD₅₀

determination. The results of the tests were reproducible and correlate well with the classical LD₅₀ values.

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