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The effect of chloroquine chemoprophylaxis on workers in Kazunma area

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The study was conducted in November 1990 in the Kazunma village area of the Seikphyu Township. The study included 300 villagers of the Kazunma village as the control and the 315 workers of the Kazunma camps. The workers were on chloroquine chemoprophylaxis for more than three months. Among the villagers of the Kazunma village 16% were infected with malaria, *P. falciparum* 64.8% and *P. vivax* 31.3%. The malaria infection rate of the workers from the northern and the base camps were similar to the villagers. The workers from the southern camps have higher level of parasitaemia (26%) inspite of the chemoprophylaxis. Though the chemoprophylaxis has no effect on the parasite rate, it seems to have some effect on the parasite density of the workers of the northern and the base camps. The *in vitro* sensitivity of *P. falciparum* isolates were 30.8% sensitive to chloroquine and 55.6% sensitive to amodiaquine.

INTRODUCTION

According to VBDC report 8.6% of the total population are residing in malaria free area and 36.9% are in high malaria prevalence area in Myanmar. At present various groups of non immune and semi-immune workers are assigned in the malaria endemic areas (such as oil drilling irrigation, wood cutting, etc.). The chemoprophylaxis is the only mean of malaria control among them. This study was intended to find out the malaria situation of the area and to determine the effect of chloroquine (300 mg base weekly) chemoprophylaxis as a control measure in the area.

MATERIAL AND METHOD

Area and study population

The study was conducted in Kazunma

village, Seikphyu Township which is in the western side of the Ayeyarwady river in the middle part of Myanmar. The village is in the plain area and is about 7 kms away from the mountain ranges. The base camp is 3 kms away from the village.

The study population included the workers (mainly recruited from the Township) of the Kazunma oil drilling camps and of the villagers in the Kazunma village.

Chemoprophylaxis and treatment

The workers of the camps were on regular chloroquine chemoprophylaxis at 300 mg base weekly for more than 3 months. The drugs were supplied through the supervisors. The villagers are not on chemoprophylaxis. There is a Station Hospital in the village and a medical team, headed by a doctor in the camp for treatment of malaria cases.

Blood film examination

Thick films were collected from the finger-tip blood. Staining was done with 10% Giemsa stain for 30 min and examined for 20 thick fields for each slide.

In vitro drug sensitivity

In vitro drug sensitivity was done by using the WHO precharged plates. The patient selection criteria was also done by using the WHO in vitro criteria (WHO, 1978).

RESULTS

Malaria situation of Kazunma village

The main type of work in the village is farming, wood and bamboo cutting in the deep forest and mountain ranges.

Out of the 300 villagers of the Kazunma village, 16% were infected with malaria of which P. falciparum constitutes 64.6% and P. vivax 31.3% (Table 1).

The spleen rate among 2-12 years old children was 29% with the average enlarge spleen of (1.3%) [1].

The malaria situation of the camps

There were (964) staffs in the camps.

Base camp

Out of the (157) blood slides taken from the workers (17.8%) were infected. Out of them 75% were P. falciparum and 25% were infected with P. vivax (Table 1).

Northern and southern camps

The blood films taken from 85 workers from the northern camp showed 14 (i.e., 16.3%) malaria positive cases with 86.7% P. falciparum and 13.3% P. vivax cases.

The examination of the (73) blood slides from the southern sector of the area showed, malaria infection rate of 26% with P. falciparum and P. vivax rate of 68.4% and 31.5% respectively (Table 1).

Table 1. Malaria infection rate of the villagers and the workers of the area

No.	The study area (on chloroquine chemoprophylaxis)	No. Examined	No. Positive cases (%)	MALARIA Species		
				<u>P. falciparum</u>	<u>P. vivax</u>	MIX Pf + Pv
1.	SSL (Base camp)	157	28 (17.8%)	21 (75%)	7 (25%)	-
2.	Southern part of base camp	73	19 (26%)	13 (68.4%)	6 (31.6%)	-
3.	Northern part of base camp	85	14 (16.5%)	12 (85.7%)	2 (14.2%)	-
	Total	315	61 (19.4%)	46 (75.4%)	15 (24.5%)	-
=====						
4.	Kazunma village (villagers - control)	300	48 (16%)	31 (65%)	15 (31.3%)	2 (4.2%)

Table 2. In vitro sensitivity of Plasmodium falciparum isolates to antimalarials

CHLOROQUINE				AMODIAQUINE				SULPHADOXINE PYRIMETHAMINE				QUININE				MEFLOQUINE			
DILUTION OF DRUG		CUMULATIVE NUMBER		DILUTION OF DRUG		CUMULATIVE NUMBER		DILUTION OF DRUG		CUMULATIVE NUMBER		DILUTION OF DRUG		CUMULATIVE NUMBER		DILUTION OF DRUG		CUMULATIVE NUMBER	
P mol/well		% INHIBITION		P mol/well		% INHIBITION		P mol/well		% INHIBITION		P mol/well		% INHIBITION		P mol/well		% INHIBITION	
1	0/26(0%)	0.25	0/18(0%)	10	0/28(0%)	4	0/24(0%)	2	6/21(29%)										
2	0/26(0%)	0.5	0/18(0%)	30	0/28(0%)	8	6/24(25%)	4	15/21(71%)										
4	2/26(8%)	1.0	1/18(6%)	100	6/28(21%)	16	2/24(8%)	8	20/21(95%)										
8	8/26(31%)	2.0	10/18(56%)	300	24/28(86%)	32	10/24(42%)	16	21/21(100%)										
16	12/26(62%)	4.0	12/18(67%)	1000	25/28(89%)	64	15/24(63%)	32	100%										
32	17/26(65%)	8.0	13/18(72%)	3000	26/28(93%)	128	22/24(92%)	64	100%										
64	23/26(88%)	16.0	18/18(100%)	10000	28/28(100%)	256	24/24(100%)	128	100%										

N.B. - Underlined showed the cut off point for sensitivity

Table 3. Comparison of parasite density in different areas

Experiment	The study area	<u>P. vivax</u>			<u>P. falciparum</u>			Total		
		+	++	+++	+	++	+++	+	++	+++
Chemoprophylaxis (workers)	Base camp	7 25%	-	-	21 75%	-	-	28 100%	-	-
	Southern camps	3 15.8%	-	-	16 84.2%	-	-	19 100%	-	-
	Northern camps	5 35.7%	2 14.3%	-	7 50%	-	-	12 85.6%	2 14.4%	-
	Total	15 24.6%	2 3.3%	-	44 72.1%	-	-	59 96.7%	2 3.3%	-
	Kazunma village	15 31.3%	-	-	24 50%	6 12.5%	1 2.1%	43 89%	6 12.5%	1 2.1%
Control (villagers)										
(MIX 4.2)										

Grading + parasite 1 to 5/oil field
 ++ parasite 5 to 20/oil field
 +++ parasite 20 and above/oil field

In vitro drug sensitivity

The sensitivity of P. falciparum to antimalarial was tested by in vitro culture of (36) isolates obtained from the patients by using WHO test kits.

The P. falciparum isolates were 100% sensitive to quinine and mefloquine. They were 30.8% sensitive to chloroquine and 55.6% sensitive to amodiaquine. The isolate were 85.7% sensitive to sulphadoxine/pyrimethamine (Table 2).

DISCUSSION

Malaria transmission is present in the Kazunma village area during the study period. The P. falciparum isolates in the area, were more sensitive to the antimalarials, than the isolates from other areas in Myanmar. They are only 69% resistance to chloroquine (Table 2).

The P. falciparum isolates in the Mawlamyaing and Lashio areas in 1990 study, showed 95% to 100% resistance to chloroquine. However the isolates in Kazunma showed 69% resistant to it.

Though the P. falciparum isolates in Kazunma were more sensitive to chloroquine than the other areas in Myanmar, the parasite rate of the workers in the base and northern camps (17.8% and 12.5%) were similar to the unprotected villagers. The parasite rate of the workers in the southern camp (26%) was even much higher than the villagers.

The rate of fever cases among the workers (14.3%) were not significantly different from that of the villagers (11.7%). It seemed that the chloroquine chemoprophylaxis had no effect on the parasite rate nor on the clinical attacks of malaria. However the parasite density was significantly lower in workers of

the base and southern camps than the villagers (Table 3). Chloroquine prophylaxis seemed to have some effect on the density of parasitaemia in low level parasite density area.

Though chloroquine has been reported to cause severe itching [3], we did not find severe reactions in our study.

In conclusion, chloroquine chemoprophylaxis is not advisable for the chloroquine resistant rate is very high in Myanmar (95 to 100%) [4] and there is the danger of emergence and spread of chloroquine resistance strains by chloroquine chemoprophylaxis [2,5]. Also the present study showed unfavourable results of the chloroquine chemoprophylaxis.

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Utilization of gynaecologic beds at
at the Central Women's Hospital (1990)

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A one year situational analysis of utilization of gynaecological beds at the Central Women's Hospital (Unit II) is presented. The pattern of admission, details of treatment, duration of hospital stay and bed occupancy were determined. There were 667 admissions of which 564 were elective admissions and 103 were emergencies. Of all admissions, 84.1 percent were operated on. Of the abdominal operations, hysterectomy (135) was the most common operation followed by female sterilization (120). Anterior and posterior colporrhaphy (56) was the commonest vaginal operation performed. The mean hospital stay for cases of female sterilization, myoma uterine, ovarian tumour and uterovaginal prolapse was 7 days, 26 days, 22 days and 19 days respectively while the mean waiting time was 5 days, 14 days, 14 days and 11 days respectively. Recommendations whereby the waiting time and the hospital stay and indirectly the bed occupancy can be reduced are presented. It is hoped that quality medical care can be rendered by optimal use of existing resources.

INTRODUCTION

Owing to the rising costs of services, health administrators suggest that rational allocation and efficient use of resources may curtail unnecessary costs [1]. The important role of health economics in developing countries has been underscored [2]. Studies have been conducted to determine the efficiency with which hospital beds are used.

The Central Women's Hospital is a teaching hospital (tertiary centre) for medical care in Obstetrics and Gynaecology. It receives both elective and emergency obstetric and gynaecological patients. There are

three units in the hospital offering medical services to in-patients. This study addresses the efficiency with which hospital beds were used by one of the units in Central Women's Hospital. A one year situational analysis of utilization of existing resources by one of the hospital units is presented.

Objectives

The objectives of the study are:-

1. To determine the pattern of gynaecological admissions to a Unit (Unit II) in Central Women's Hospital.
2. To review the different types of operative and non-operative forms of treatment offered to

- gynaecological in-patients.
3. To determine the basic hospital performance indices of the gynaecological unit including bed occupancy and mean hospital stay.
4. To analyse the time spent on the waiting list for commonly performed gynaecological operations.
5. To offer suggestions based on these observations to improve the quality of care with the existing resources.

PATIENTS AND METHODS

The study period was a one year period from 1st January 1990 to 31st December 1990. The gynaecology ward (Unit II) log book was used to obtain details of all admissions. The details of treatment and duration of hospital stay were determined from the medical records. The length of hospital stay was defined as the duration in days from admission to discharge. Bed occupancy is the number of bed days which each patient used. The occupancy rate is defined as the percentage of occupancy (bed-day) of each condition divided by the total number of bed days in the ward. The time spent on the waiting list is the time from admission to the day before the operation.

The methods of treatment were divided into two main groups, operative and non-operative, of both the elective admissions and the emergency.

Operative procedures were further subdivided into abdominal and vaginal operations. In non-operative treatment, the patients had either chemotherapy, radiotherapy, medical or conservative treatment.

RESULTS

There were 667 admissions during the one year period with an average of 55.5 admissions per month. Of these, 564 (84.6 percent) were elective admissions and 103 (15.4 percent) were admitted for emergency conditions. Of all the admissions, 84.1 percent were operated and 15.9 percent were not operated.

The pattern of gynaecological admissions are shown in Table (1). Admission for female sterilization

Table 1. Pattern of gynaecological admissions

Diagnosis	Number	Percent
For female sterilisation (interval)	132	20.4%
Myoma uterus	107	16.6%
Uterovaginal prolapse	101	15.6%
Ovarian tumours	90	13.9%
Abnormal uterine bleeding	43	6.6%
Ectopic pregnancy	35	5.4%
Carcinoma cervix	23	3.6%
Gestational trophoblastic disease	20	3.1%
Others	97	14.9%
Total	647	100.00
Readmissions	20	
	667	

topped the list followed by myoma uterus, uterovaginal prolapse and ovarian tumours. This pattern has been seen in other data available from Central Women's Hospital [3,4].

In Table (2), the detailed breakdown of commonly performed abdominal operations is presented. Hysterectomy (with or without adnexal operations) was the commonest abdominal operation per-

Table 2. Operative treatment

Category	Number of cases	Mean hospital stay	Occupancy rate
<u>Abdominal operations</u>			
Hysterectomy ± adnexal operation	135	24.55	22.51
Female sterilisation	120	7.8	5.75
Adnexal operation	32	25.5	5.59
<u>Vaginal operations</u>			
A C - P C P	56	18.7	7.17
Vag. hysterectomy and repair	25	22.45	3.84
Manchester repair	12	19.9	1.64
B & C	32	8.0	

formed. This was followed by female sterilisation.

Among the vaginal operations, anterior and posterior repair was the commonest operation performed. The table also shows the mean hospital stay and occupancy rate of each of the conditions.

Among the different types of non-operative treatment, chemotherapy-42 patients (40 percent) and conservative management of gynaecological bleeding 37 patients (35 percent) were the two commonest types of treatment offered. There were 24 admissions of malignant ovarian tumour, 13 of gestational trophoblastic disease and 5 admissions of carcinoma cervix for chemotherapy. Other forms of non-operative treatment included medical treatment, radiotherapy and supportive measures.

Surgery for ectopic pregnancy was the commonest operation performed (44.3%) amongst 79 emergency operations. This was followed by surgery for uterine rupture and perforation

(12.7%). Although uterine rupture is an obstetric emergency, in the post-operative period the patients are kept in the gynaecological ward and occupy gynaecological beds. Repair of vaginal tears (11.4%) is the next commonly encountered gynaecological emergency. Others include drainage of pelvic abscess, drainage of haematocolpos and ovariectomy.

Regarding management of ectopic pregnancy, 24 out of 35 cases (68.5 percent) were operated immediately. Seven cases were operated within one week and four cases within two weeks. In cases where the diagnosis was not immediately established, there was a delay in laparotomy leading to an increased length of hospital stay.

There is an intensive care unit which is common to all gynaecological units. There were four admissions to the unit, three were cases of respiratory and cardiac arrests following anaesthetic accidents. One was a patient with adult respiratory distress following eclampsia. All the cases survived with no residual handicap.

In Table (3), further analysis of bed occupancy is presented. The mean waiting time for common gynaecological conditions are presented. On an average, admissions for female sterilisation had to wait five days, patients with myoma uterus and ovarian tumours for two weeks and patients with uterovaginal prolapse for eleven days. Further analysis of waiting time showed that a small number of cases with a prolonged waiting time were responsible for the increase in the length of hospital stay. This leads to an unnecessary increase in bed occupancy by in-patients.

Table 3. Mean hospital study and mean waiting time for common gynaecological conditions

Gynaecological condition	Mean waiting time (days)	Mean hospital stay (days)
Admissions for female sterilisation (interval)	5.34	7.00
Myoma uterus	14.98	26.77
Ovarian tumour	14.7	22.8
Utero-vaginal prolapse		
for A.C. and P.C.P.	10.36	18.7
for Manchester repair	11.91	19.9
for vaginal hyst	12.44	22.45
for P.C.P.	8.78	15.5

In Table (4), comparison of bed utilization at Central Women's Hospital and Ramathibodhi Hospital [5] is presented. As data of only one out of three gynaecological units was analysed, the total number of cases and the number of operative cases was less in Central Women's Hospital. In general, although the bed occupancy was similar, the mean hospital stay was found to be three of four times more in Central Women's Hospital.

DISCUSSION

This is the first attempt to audit the use of gynaecologic beds at the Central Women's Hospital. It is hoped that this baseline data will serve to improve administrative and clinical services rendered.

The total bed occupancy rate was 75 percent. This indicates the work load done by a hospital [6]. However, a prolonged period of time spent on the waiting list is partly responsible for the occupancy rate. If the duration of time spent on the waiting list could be reduced, it

Table 4. Comparison of gynaecological bed utilization at Central Women's Hospital (Unit II) with Ramathibodhi Hospital (duration of study - one year)

	Central Women's Hospital (2) No. of beds 40	Ramathibodhi Hospital No. of beds 43
Total admissions	667	1545
Operative treatment	84.1%	72%
Hysterectomy		
Number of cases	135	499
Mean hospital stay	24.55	7.4%
Bed occupancy	22.51	23.4%
Adnexal operations		
Number of cases	32	188
Mean hospital stay	25.5	7.1
Bed occupancy	5.59	8.5
Vaginal operations		
A.C. & P.C.P.		
Number of cases	56	160
Mean hospital stay	18.7	6.9
Bed occupancy	7.17	7.0
Vaginal hysterectomy		
Number of cases	25	53
Mean hospital stay	22.44	7.6
Bed occupancy	3.84	2.6

would lead to more effective utilization of available hospital beds.

There are several reasons why time spent on the waiting list after hospitalization can be prolonged. Although the Central Women's Hospital is situated in Yangon, admissions are from all over the country. Sometimes patients have to be admitted for social reasons before pre-operative investigations can be done on an out-patient basis as is the hospital

policy. Also, as the Central Women's Hospital is a tertiary care hospital, it is a receiving centre for complicated cases who require sophisticated investigations and management. Cases may also have general medical complications and require the combined care of physician-gynaecologist, surgeon-gynaecologist or oncologist-gynaecologist teams. Patients may have anaesthetic risks and require special anaesthetic services and care. Cases admitted for interval sterilization may appear to have as unduly long waiting time. This is because post-partum sterilization cases are given preference over interval sterilization.

The following suggestions are made to prevent an unnecessary waiting period pre-operatively.

1. A thorough clinical work-up at the out-patients department to reach a correct diagnosis.
2. Where the diagnosis is in doubt, to perform relevant ancillary investigations to support the diagnosis; wherever possible, as an out-patient procedure. To consult a colleague or consultant for a review of the case and a second opinion.
3. Where general medical problems are found to co-exist, to refer the patient for anaesthetic and medical consultation prior to admission for necessary specialist care.
4. To give the patient a realistic and feasible admission date, taking into consideration the current status of the in-patients who have already been warded.
5. In some cases (30%) of ectopic pregnancy, the diagnosis was delayed. The use of diagnostic laparoscopy in the management of the suspected ectopic is strongly advocated.
6. From the point of view of the

Health Services, if the general population is made aware of the specialist services that are available at State/Divisional Hospitals, the workload on the Central Women's Hospital could be reduced so that it could truly function as a tertiary care centre.

It is hoped that the above suggestions based on the administrative audit will help to streamline and maximise the use of medical resources so that more efficient and cost-effective medical care can be given.

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Antibacterial activity of honey, propolis and bee pollen: an in-vitro study

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An in-vitro study was carried out to determine the antibacterial activity of honey, propolis (bee glue) and bee pollen. It was found that pure honey is a potent inhibitor of all the pathogens tested. Most pathogenic bacteria (except Salm. enteritis and enteropathogenic Escherichia coli) failed to grow in honey at a concentration of 30 per cent and above. Propolis was found to have antibacterial activity only against gram-positive cocci, but limited activity against gram-negative bacilli. Propolis can eliminate the growth of gram-positive cocci even at a concentration of 10 per cent. Bee pollen has no antibacterial effect on all the pathogens tested. The findings confirm previous reports of antimicrobial properties of these materials.

INTRODUCTION

Honey, propolis (bee glue) and bee pollen has had a valued place in traditional medicine for centuries. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut. In the past, scientific opinions on honey's nutritive and medical uses have been differed and clashed with folklore. Controversies within the scientific community have interest in the therapeutic uses of honey in modern medicine.

The anti-infective action traditionally attributed to honey and other bee products in folk medicine seems to have a sound scientific base according to research by microbiologists [1,2]. Although a vast number of papers and articles have been published on the subject, most have concentrated on the biochemical analysis and food and non-food commercial uses. Recently, scientific support has emerged with a proliferation in publications on

the successful therapeutic use of honey and bee products in several general medical and surgical conditions. A number of workers have reported the antibacterial effect of pure honey and other bee products [1,3]. The therapeutic potential of pure honey is grossly underutilized. As it is easily available in most communities of Myanmar, some medicine containing honey and other bee products have been manufactured for curing some ailments like intestinal disorders, diseases of liver, upper respiratory tract infections, wounds and ulcers.

So far, no scientific investigation have been done on Myanmar bee products, although the mechanism of action of several components of its properties remains obscure and needs further investigation. So, it is the time for conventional medicine to lift the blinds off this traditional remedy. It will be tremendous assistance in determining the therapeutic properties especially antibacterial activity.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains included in this study were pathogenic Escherichia coli, Salmonella typhimurium, Salmonella enteritidis, Shigella dysenteriae, Vibrio cholerae, Non O1 Vibrio, Aeromonas hydrophilia and gram-positive cocci like Staphylococcus aureus and B. hemolytic Streptococci.

Preparation of bacterial strains

Bacterial strains to be tested were cultured in Trypticase Soy broth (1 ml) (TSB, BBL) and incubated at 37° Celcius for 18-24 h. On the next day, bacterial suspension were diluted with sterile distilled water to provide appropriate concentration (10^8 CFU/ml).

Preparation of honey and bee products

Honey and bee products employed in this study were kindly provided by Bee Keeping Department of Myanmar through Department of Traditional Medicine. Natural condition of honey had been previously screened for any contamination. Propolis and bee pollen suspended in sterile distilled water at 3 per cent (W/V) concentration were mixed thoroughly and held at room temperature for 3 h and then autoclaved at 121° Celcius for 15 min.

Preparation of solid agar plate media

- (1) Control plates were prepared according to the manufacturer's instruction using Trypticase Soy

agar (BBL).

- (2) Honey agar plates were prepared with varying concentration of honey (10-50%) added to trypticase Soy Broth (BBL) containing 1.5 per cent agar medium at 50° Celcius and then poured into the petri-dishes.
- (3) Propolis and bee pollen agar plates were made by using sterile 3 per cent solution of propolis and bee pollen prepared as described above. Varying concentration of respective solution were added to TSB containing 1.5 per cent agar medium and poured into petri-dishes.

After cooling and drying, the plates were inoculated with bacterial suspension using calibrated wire loop. In each case, 0.01 ml of bacterial suspension was delivered to the agar surface. Then the plates were incubated aerobically for 18-24 h at 37° Celcius and observed the growth.

Detection of bactericidal activity

To determine whether the observed inhibition was bactericidal or bacteriostatic, plate count method was used. Three ml of pure honey was mixed thoroughly with 20 ul of bacterial suspension (10^8 CFU/ml) prepared as described before. After incubation at 37° Celcius, passages were made onto Nutrient Agar (Difco) plates at daily intervals up to 7 days. Bactericidal action of propolis was also carried out using the same method. Experiments were done for 3 times for each cases.

RESULTS

There was good growth of all bacteria tested on the control plates. Table 1 shows that all intestinal

Table 1. Growth of bacteria on plates containing varying concentration of honey, propolis (bee glue) and bee pollen

Pathogens	Growth on control plates	Conc. of honey					Conc. of propolis					Conc. of bee pollen 50%
		10%	20%	30%	40%	50%	10%	20%	30%	40%	50%	
1. <u>Salm. enteritis</u>	+	+	+	±	-	-	+	+	+	+	+	+
2. <u>Salm. typhimurium</u>	+	+	+	-	-	-	+	+	+	+	+	+
3. <u>Aeromonas spp.</u>	+	+	+	-	-	-	+	+	+	+	+	+
4. <u>S. dysenteriae</u>	+	+	+	-	-	-	+	+	+	+	+	+
5. <u>Pathogenic E. coli</u>	+	+	+	±	-	-	+	+	+	+	+	+
6. <u>V. cholerae</u>	+	+	+	-	-	-	+	+	+	+	+	+
7. <u>Non O1 Vibrio</u>	+	+	+	-	-	-	+	+	+	+	+	+
8. <u>Staph. aureus</u>	+	+	+	-	-	-	-	-	-	-	-	+
9. <u>Streptococcus</u>	+	+	+	-	-	-	-	-	-	-	-	+

Note: -, no growth; +, growth; ±, scanty growth

Table 2. Bactericidal effect of honey and propolis against pathogenic bacteria

Bacterial strains	Results of daily bacterial investigation													
	honey							propolis						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1. <u>Salm. enteritidis</u>	+	+	+	-	-	-	-	+	+	+	+	+	+	+
2. <u>Salm. typhimurium</u>	+	+	-	-	-	-	-	+	+	+	+	+	+	+
3. <u>Aeromonas spp.</u>	+	+	+	-	-	-	-	+	+	+	+	+	+	+
4. <u>S. dysenteriae</u>	+	+	-	-	-	-	-	+	+	+	+	+	+	+
5. <u>Pathogenic E. coli</u>	+	+	-	-	-	-	-	+	+	+	+	+	+	+
6. <u>Vibrio cholerae</u>	+	-	-	-	-	-	-	+	+	+	+	+	+	+
7. <u>Non O1 Vibrio</u>	+	-	-	-	-	-	-	+	+	+	+	+	+	+
8. <u>Staph. aureus</u>	+	+	-	-	-	-	-	+	+	-	-	-	-	-
9. <u>Streptococcus</u>	+	+	-	-	-	-	-	+	-	-	-	-	-	-

pathogens tested failed to grow in honey at a concentration of 40 per cent and above. Gram-positive cocci can not grow in honey at a concentration of 30 per cent and above.

Propolis can only inhibit the growth of gram-positive cocci namely Staphylococcus aureus and hemolytic Streptococci even at a concentration of 10 per cent. In other words, it has no antibacterial effect on intestinal pathogens. Bee pollen was found to be not effective on all the pathogens tested.

Table 2 shows that all the bacterial pathogens tested failed to grow in pure honey within 1 and 3 days after incubation. As shown in this table propolis is not so effective to inhibit the growth of enteric pathogens. But it can eliminate the growth of gram-positive cocci such as Staphylococcus aureus and hemolytic Streptococci within 2 days and 1 day respectively.

DISCUSSION

The results of this study confirms

the antibacterial effect of honey on various gram-positive and gram-negative bacteria. In particular, pure honey is a very potent inhibitor of the growth of bacteria such as enteropathogenic *Escherichia coli*, *Aeromonas*, *Salmonella*, *Shigella*, *Vibrio cholerae*, Non O1 *Vibrio*, all of which are known to cause considerable morbidity and mortality from diarrhoeal and dysenteric diseases in developing countries.

The mechanism of antibacterial effect of honey remains speculative at present. Possible explanations are shrinkage disruption of the bacterial cells because of the osmotic effect of the hypertonic sugar concentration in honey, the low pH or the presence of some substances in honey with bactericidal properties. However, antibacterial effect is due to the combined action of several components present in honey.

The results also shows that propolis has bactericidal activity only against the commonly encountered cocci but no activity against gram-negative bacteria. This fact is also in agreement with the findings of other scientists [1]. They found that propolis had antimicrobial activity against a range of commonly encountered cocci and gram-positive rods, including the human tubercle bacillus, but only limited activity against gram-negative bacilli. Unfortunately, antibacterial action of honey and other bee products against tubercle bacillus was not determined in this study.

The nature of the antibacterial components of propolis has not been elucidated but evidence points to the flavenoid content (which are known to have many biological

effects in animal systems) and various esters of caffeic acid [1]. In addition to antibacterial effect, propolis may have antiviral, anti-protozoal and anti-dermatophyte actions. Some researchers also reported that, extracts of propolis are non-toxic in experimental animals, and have had limited use in humans as aerosols, eye drops and hand disinfectants. In this study, the components of honey and other bee products, responsible for the observed bactericidal activity was not identified. It seems likely that the beneficial effects of propolis and honey are the results of their flavenoid content, and both of these natural compounds deserve further appraisals of their therapeutic efficacy. Therefore, it is suggested for further evaluation on clinical application of honey for gastroenteritis and various wounds and ulcers. As propolis is found to be effective on gram-positive cocci, it should be considered for use in the treatment of wounds and ulcers as well as upper respiratory tract infections.

The results showed that bee pollen was not effective in inhibiting the growth of both gram-positive and gram-negative bacilli. This may be due to low concentration of pollen contained in the culture medium. Owing to the different origin and composition of pollen and the method of investigation, the results of antibacterial action of pollen are sometimes inconsistent. Therefore, further investigation of antibacterial activity of pollen may be the subject of continual study to draw a constant conclusion. Ioyish [4] had found out the fact that bee pollen is effective in treating the intestinal disorders and diseases of liver, endocrine systems etc. But no one can explain the mechanism of action of these effects.

Therefore, in addition to the antibacterial property, investigation of other properties of honey and bee products is necessary. To know the mechanism of action of various properties present in these substances, it is also essential to identify these various components responsible for such activity.

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Quantitative determination of Senna ingredient from
Traditional Medicine Formulations - TMF-09, TMF-10
and TMF-12

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Quantitative determination of a major plant ingredient Senna contained in Myanmar Traditional Medicine Formulations (TMFs) - namely TMF-09, TMF-10 and TMF-12 were estimated by following Schedule XIII and XIV of Wallis (1953). Quantitative microscopic work conducted at the Pharmacognosy Laboratory of the Pharmacology Research Division, could quantify the proportion of Senna ingredient, from the above three TMFs.

INTRODUCTION

Quantitative microscopic analysis methodology was introduced by Wallis [1] using the Lycopodium technique, quantitative schedule XIII. This method enables to determine the percentage purity, authenticity, adulterants and substitutes of any single plant drug. Proportion of any plant ingredient in a compound formulation (powder mixture) can also be estimated by another quantitative schedule XIV. The proportion of plant ingredients in a given formulation can be estimated, if there are four or less ingredients, as well as a major ingredient that can be easily differentiated from the remaining plant ingredients. It is of interest, whether the plant ingredient or ingredients contained in the Myanmar TMFs can be quantitated by applying the schedule XIII and XIV.

No quantitative estimation of the plant ingredients contained in the Myanmar TMFs has been conducted so far. TMF-09, TMF-10 and TMF-12 were selected for the present study, since

Senna is one of the major ingredient and the characters of Senna can also be easily differentiated from the remaining plant ingredient characters.

This study was undertaken to establish a practical microscopic quantitative method in quantifying the proportion of plant ingredient from the TMFs.

MATERIALS AND METHODS

Materials

Prepared traditional medicine formulations: -TMF-09, TMF-10 and TMF-12 as shown in table 1, 2 and 3 [2] and air dried Senna leaflets undergoing investigation were supplied by the manufacturers of the production unit from the Department of Traditional Medicine (DTM), Yangon.

Methods

1. Preparation of air dried Senna leaflets powder

Table 1. Ingredients of TMF-09 (HSEEDULAR HSAY)

Name of Ingredient		Weight		Amount in g per 100 g
Myanmar	Scientific or Common	Myanmar Kyat Pe	Metric g	
<u>Plant origin</u>				
1. Alokyu (Rhizome)	<u>Arundo donax</u> Linn.	1 -	16	4.5
2. Nweigyo (Stem)	<u>Glycyrrhiza glabra</u> Linn.	1 -	16	4.5
3. Kyazoot (Fruit)	<u>Terminalia citrina</u> Roxb.	1 -	16	4.5
4. Mingogar (Stem)	<u>Argyrea barbigera</u> Choisy.	3 -	448	13.6
5. Pwaygaing (Leaf)	<u>Cassia angustifolia</u> Vahl.	8 -	128	36.4
<u>Chemical Origin</u>				
6. Hsaboks	Black salt*	2 -	32	9.1
7. Theindaw	Natural sodium chloride; rock salt	2 -	32	9.1
8. Zawetthar	Ammonium chloride	2 -	32	9.1
<u>Animal Origin</u>				
9. Pinleiyayhmyoke	<u>Sepia esculenta</u>	- 8	8	2.3
10. Kywayboke	<u>Cypraea eglantina</u>	- 8	8	2.3
11. Leikkyaukhsou	<u>Trigon zeygi</u>	- 1	16	4.5

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

Senna leaflets from DTM were pulverized by using model type No. 3, Wiley Grinding Mill (Arthur H. Thomas, Philadelphia, U.S.A.) and then sieved successively through 2mm and then to 1mm mesh sieves and finally through 0.14 mm sieve by the model EML 200 laboratory sieve shaker (Haver and Boecker, Oelde, West Germany).

Powdered Senna was weighed with Jupiter CT3-200D electrical balance (Chyo, Japan) for quantitative determination of authentic study.

2. Identification of diagnostic particles of Senna powder

A microscopic examination of the diagnostic features of the leaf powder was characterized by clearing in solution of chloral hydrate and glycerol (9:1). The characters were documented by line drawing with a

magnification of 3150 times using (10x eyepieces, 40x objective and 7.875x drawing tube).

3. Quantitative determination of the number of spores per milligram of lycopodium

Method was employed according to quantitative schedule XIII by Wallis [1].

4. Quantitative determination of area per milligram of sieved authentic Senna powder

Quantitative schedule XIV by Wallis [1] was slightly modified regarding the preparation of suspension ratio of 3:2 glycerol in chloral hydrate and area of the diagnostic particle was evaluated instead of counting the numbers. The particle index (PI) values were used as standard for quantitation of the TMFs and

Table 2. Ingredients of IMF-10 (HSEESWIN WUNHNOKE HSAY)

Name of Ingredient		Weight		Amount in g per 100 g
Myanmar	Scientific or Common	Myanmar Kyat Pe	Metric g	
<u>Plant Origin</u>				
1. Zardeikpho thee (Fruit)	<u>Myristica fragrans</u> Houtt.	- 8	8	0.5
2. Zardeikpho bwint (Aril)	<u>Myristica fragrans</u> Houtt.	- 8	8	0.5
3. Peikchin thee (Fruit)	<u>Piper longum</u> Linn.	- 8	8	0.5
4. Gyngyauk (Rhizome)	<u>Zingiber officinale</u> Roscoe.	- 8	8	0.5
5. Eikmwayt thee (Fruit)	<u>Embellia robusta</u> Roxb.	- 8	8	0.5
6. Ihinbawseinnabaw (Rhizome)	<u>Smilax</u> sp.	- 8	8	0.5
7. Kaleinzeet (Seed)	<u>Caesalpinia bonducella</u> Flem.	- 8	8	0.5
8. Chee thee (Fruit)	<u>Semicarpus anacardium</u> Linn. f.	- 8	8	0.5
9. Kalaw zan (Seed)	<u>Hydnocarpus kurzii</u> (King) Warburg	- 8	8	0.5
10. Hsinnamoung (Whole plant)	<u>Heliotropium</u> sp.	1 -	16	1.0
11. Alokyu (Rhizome)	<u>Arundo donax</u> Linn.	1 4	20	1.2
12. Nweigyo (Stem)	<u>Glycyrrhiza glabra</u> Linn.	2 8	40	2.4
13. Kyazoot (Fruit)	<u>Terminalia citrina</u> Roxb.	12 8	200	11.9
14. Mingogar (Stem)	<u>Argyrea barbigera</u> Choisy.	12 8	200	11.9
15. Pwaygaing (Leaf)	<u>Cassia angustifolia</u> Vahl.	36 -	576	34.2
<u>Chemical Origin</u>				
16. Kyaukchin	Potassium aluminium sulphate; alum	- 8	8	0.5
17. Hsaboke	Black salt*	5 -	80	4.8
18. Zawetthar	Ammonium chloride	12 8	200	11.9
19. Theindaw	Natural sodium chloride; rock salt	15 -	240	14.3
<u>Animal Origin</u>				
20. Leikkyaukhsou	<u>Trigon zeygi</u>	1 -	16	1.0
21. Kywayboke	<u>Cypraea eglantina</u>	1 -	16	1.0

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

expressed as deduced from the following formula according to:

$$PI_{(area)} = \frac{a \times w \times LF}{s}$$

where $PI_{(area)}$ = relative area of test drug particles per lycopodium spore per mg of the drug

a = area of characteristic particle (sq mm) in seven strips

s = number of spores in twenty five fields

w = weight in mg of lycopodium taken

LF = lycopodium factor (94,000 spores per mg of lycopodium)

Table 3. Ingredients of TMF-12 (SETKHUPALA HSAY)

Name of Ingredient		Weight		Amount in g per 100 g
Myanmar	Scientific or Common	Myanmar Kyat Pe	Metric g	
<u>Plant Origin</u>				
1. Karaway ywet (Leaf)	<u>Cinnamomum</u> sp.	- 2	2	0.2
2. Pharlarngei (Seed)	<u>Elettaria cardamomum</u> Maton. var <u>miniscula</u>	- 2	2	0.2
3. Chee thee (Fruit)	<u>Semicarpus anacardium</u> Linn.	- 4	4	0.4
4. Pannoot (Root)	<u>Saussurea</u> sp.	- 4	4	0.4
5. Zardeikpho thee (Fruit)	<u>Myristica fragrans</u> Houtt.	- 4	4	0.4
6. Layhnyin (Flower)	<u>Syzygium aromaticum</u> (Linn.) Merr. & Perry	- 4	4	0.4
7. Kalaw thee (Fruit)	<u>Hydnocarpus kurzii</u> (King.) Warburg	- 4	4	0.4
8. Bomayarzar (Root)	<u>Rauwolfia serpentina</u> Benth.	1 -	16	1.7
9. Kantgyokni (Stem)	<u>Plumbago rosea</u> Linn.	1 -	16	1.7
10. Peikchin thee (Fruit)	<u>Piper longum</u> Linn.	1 -	16	1.7
11. Hsaypale (Root)	Unidentified	1 -	16	1.7
12. Samonnet (seed)	<u>Nigella sativa</u> Linn.	1 -	16	1.7
13. Maniawga (Bark)	<u>Carallia brachiata</u> (Lour.) Merr.	1 -	16	1.7
14. Samonsabah	<u>Foeniculum vulgare</u> Mill.	- 4	4	0.4
15. Samonbyu (Fruit)	<u>Trachyspermum ammi</u> (Linn.) Sprague	- 4	4	0.4
16. Samonnyo (Fruit)	<u>Anethum sowa</u> Roxb.	- 4	4	0.4
17. Samonphwe (Stem)	<u>Foeniculum vulgare</u> Mill.	- 4	4	0.4
18. Samonni (Seed)	<u>Lepidium sativum</u> Linn.	- 4	4	0.4
19. Pwaygaing (Leaf)	<u>Cassia angustifolia</u> Vehl.	15 -	240	25.4
20. Hsoogauknet (Bark)	<u>Capparis sepiaria</u> Linn.	20 -	320	33.8
<u>Chemical Origin</u>				
21. Letchah meebauk	Roasted borax	- 2	2	0.2
22. Hsahlaw	Roasted common salt	15 -	240	25.4
23. Sheingo	<u>Ferula asafoetida</u>	- 4	4	0.4

5. Quantitative determination of the percentage by weight of Senna ingredient in sieved and unsieved TMF-09, TMF-10 and TMF-12.

It was performed employing the method 4 by Wallis [1] of quantitative schedule XIV and the percentage calculated.

RESULTS

Diagnostic microscopical characters of authentic Senna are presented in Fig. 1. Two types of stomata, rubiaceous or paracytic (2 parallel subsidiary cell) and ranunculaceous or anomocytic (3 irregular subsidiary cell) are present on both upper and

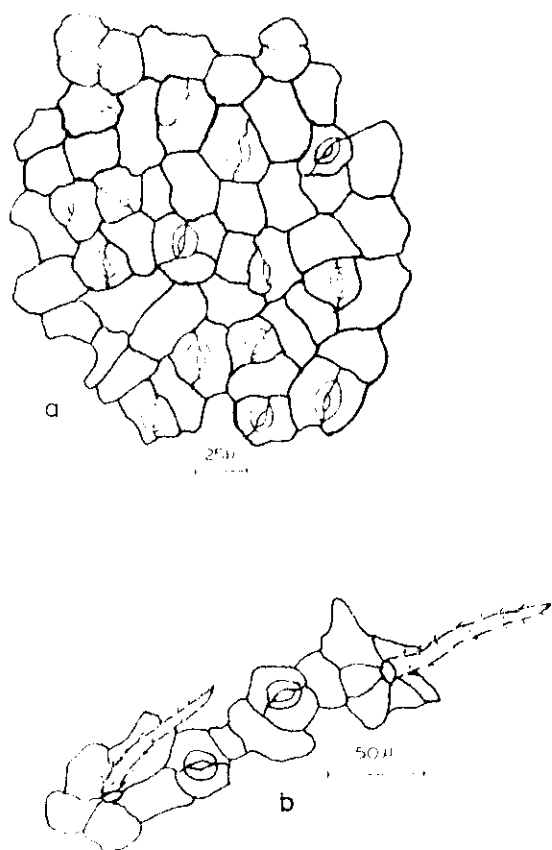


Fig. 1. a. Two types of stomata, Paracytic (parallel-celled), Anomocytic (three irregular-celled).
b. Distance between each trichome.

lower epidermi. The ratio of two subsidiary cell to three subsidiary cell is about seven to one. This character can be easily differentiated from the remaining plant ingredients of the respective TMF-09, (Plate 1), TMF-10 (Plate 1-3) and TMF-12 (Plate 1-4), published by DMR [3].

Standard authentic particle index $PI(\text{area})$ value of Senna calculated was 10.82 sq mm.

$PI(\text{area})$ and percentage values of Senna from the three TMFs are

evaluated and tabulated in table 4 and 5. In table 4, it was observed that $PI(\text{area})$ of Senna determined from the sieved TMFs were relatively

Table 4. Particle index of Senna of TMF-09, TMF-10 and TMF-12

Category	$PI(\text{area})$ of Senna (09)	$PI(\text{area})$ of Senna (10)	$PI(\text{area})$ of Senna (12)
Mixture	3.87 ± 0.18	2.74 ± 0.14	1.59 ± 0.24
Sieved			
Mixture	1.06 ± 0.26	0.68 ± 0.21	0.31 ± 0.08
Unsieved			

*denotes $p < 0.005$

Table 5. Senna percentage of TMF-09, 10 and 12

Category	Senna percentage of TMFs 09	Senna percentage of TMFs 10	Senna percentage of TMFs 12
Mixture	35.73 ± 1.62	25.34 ± 1.36	14.71 ± 2.24
Sieved			
Mixture	9.81 ± 0.98	6.25 ± 0.81	2.91 ± 0.29
Unsieved			

*denotes $p < 0.005$

higher than the unsieved TMFs. Similarly the proportion of Senna percentage estimated in table 5, from the sieved TMF-09 is almost of the same percentage as specified in table 1, whereas the values of TMF-10 and TMF-12 are relatively lower than the value specified in table 2 and table 3.

DISCUSSION

The results of $PI(\text{area})$ presented in (table 4) and proportion of Senna percentage evaluated in (table 5) shows highly significant differences between the sieved and unsieved TMFs. From these data it was shown that when employing the quantitative

method, the powder particles size of the single plant ingredients and the TMFs powder to be estimated must be of uniform size. Only then the proportion of plant ingredients contained in the TMFs can accurately be estimated.

The percentage of Senna estimated from sieved TMF-09 did not show any significant difference to that of the specified value as given in table 1. Regarding the percentage value estimated from sieved TMF-10 and TMF-12, it is significantly lower by seven and eight percent respectively to that of the value specified in table 2 and table 3. Lower percentage value estimated by this quantitative method indicates that, there is some kind of inaccuracy in the preparation.

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Antibacterial activity of bee products (bee venom & royal jelly): An in-vitro study

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Bee products namely royal jelly and bee venom provided by Bee Keeping Department of Myanmar were processed for their biological activity especially, antibacterial activity. The growth of 10 commonly encountered organisms were tested in media containing varying concentrations of bee venom and royal jelly. It was found that royal jelly and antibacterial activity on Staphylococcus aureus, Shigella dysenteriae, Aeromonas and Vibrio spp. whereas bee venom had antibacterial effect on one gram-negative bacillus (Shigella dysenteriae) and gram-positive cocci (Staphylococcus and Streptococcus spp.) tested.

INTRODUCTION

Bee products like honey, propolis (bee glue), bee pollen, royal jelly and bee venom have had their own valuable place in traditional medicine since many years ago. Scientific opinions on nutritive and therapeutic uses of these bee products have been differed and clashed with folklore in the past. Controversies within the scientific community have interest in the therapeutic uses of bee products in modern medicine. Recently, a number of workers from other countries have reported the therapeutic effects of honey [1,2,3], and propolis [4]. Although a vast number of papers and articles have been published on the subject, most have concentrated only on the biochemical analysis and commercial uses as food.

In Myanmar, antibacterial action of honey, propolis and bee pollen had been studied and reported [5]. However, it is still necessary to know whether other bee products (royal jelly and bee venom). Produced from Myanmar Bee Keeping

Department, have this effect or not. At present, there is no doubt about the curative properties of bee venom used in the treatment of some ailments of locomotion such as rheumatoid arthritis, osteoarthritis, neuralgia and myositis [6]. As far as it is concerned many aspects of the therapeutic action of bee venom are not yet studied in this country. So also, royal jelly, produced by worker bees has no proven antibacterial activity as it has been known to be rich in various vitamins and minerals.

To fulfill the knowledge of antibacterial activity of royal jelly and bee venom, an in-vitro study is carried out although the mechanisms of action of components of bee products remained obscure and needs further investigations. It is also hoped to gain tremendous assistance in determining the therapeutic action especially the antibacterial activity.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains included in this study were pathogenic Escherichia coli, Shigella dysenteriae, Salmonella typhimurium, Salmonella enteritidis, Vibrio cholerae, Non O1 Vibrio, Aeromonas hydrophilia, Proteus mirabilis and gram-positive cocci like Staphylococcus aureus and Streptococcus viridens.

Preparation of royal jelly

White and gelatinous substance, royal jelly, provided from Myanmar Bee Keeping Department was emulsified with distilled water to make varying dilutions.

Preparation of bee venom

Crude bee venom was extracted according to Langer's method [7]. The bees provided from Myanmar Bee Keeping Department were killed with chloroform and the stings were pulled out with fine pincers. Then immersed in sterile distilled water. After washing for several times, the stings were collected and crushed in 2 cc distilled water using sterile glass rod until the solution become turbid and white in colour. Then filtered and stored at 4°Celsius for the experiment.

Approximately, 3 mg of crude bee venom can be obtained from 100 bees. Various dilutions of bee venom were made in distilled water.

Detection of inhibitory activity

Disc diffusion method of Kerby and Bauer (1966) [8] was used to detect the inhibitory activity of bee products. Bacterial strains to be tested were cultured in Trypticase Soy Broth (TSB, Oxoid) and incubated

at 37°Celsius for overnight. Then washed in 1 ml Phosphate Buffered Saline (PBS) (pH 7.2) and diluted with sterile PBS to provide appropriate inoculum size (10^8 CFU per ml). Then bacterial suspensions were inoculated onto Muller Hinton (Difco, Detroit, USA) media using calibrated wire loop and disc soaked with various concentrations of bee venom and royal jelly were placed on the surface of the agar media seeded with the organism going to be examined. The plates were incubated for 24 hrs at 37°Celsius and the diameter of any resulting zones of inhibition were examined. All experiments were performed in duplicate.

Detection of bactericidal activity

Bactericidal activity of bee products were estimated by tube dilution method as described by Grange and Davey (1990) [4]. After making doubling dilutions of bee products from 1:2 in TSB media (Oxoid) and inoculating each tube with one drop (20 ul) of a bacterial suspension (10^8 CFU per ml), tubes were incubated overnight at 37°Celsius. Then loopfuls for medium were taken from each tube and streaked on TSA (Oxoid) medium to check for bacterial growth.

RESULTS

As shown in the table (Table 1) the antibacterial properties of royal jelly and bee venom (crude extract) tested by disc diffusion method were described in mm (size of clear zone). It was found that royal jelly inhibited the growth of Shigella dysenteriae, Aeromonas hydrophilia, and Vibrio spp. One of the gram-positive cocci, Staphylococcus aureus, was also inhibited by royal jelly forming clear zones

Table 1. Antibacterial effect of bee venom and royal jelly on Gram-positive and Gram-negative organisms

Organisms	Zone of Inhibition (mm in diameter)	
	Royal jelly (15%)	(100 U1%) Bee venom
1. Pathogenic <i>E. coli</i>	0	0
2. <i>Salm. typhimurium</i>	0	0
3. <i>Salm. enteritidis</i>	0	0
4. <i>Shigella dysenteriae</i>	22	25
5. <i>Aeromonas hydrophilia</i>	22	0
6. <i>Vibrio cholerae</i>	23	0
7. Non O1 <i>Vibrio</i>	20	0
8. <i>Proteus mirabilis</i>	0	0
9. <i>Staphylococcus aureus</i>	24	24
10. <i>Streptococcus viridens</i>	0	26

around the discs (between 20-24 mm in diameter). the growth of other organisms were not effected by royal jelly.

It was also noted that bee venom had inhibition effect more on the gram-positive cocci (*Staphylococcus* and *Streptococcus* spp.) than gram-negative bacilli (*Shigella dysenteriae*). No inhibition zones were observed with the growth of remaining organisms tested.

To determine the bactericidal effects, tube dilution methods was used. The results (Table 2) showed that royal jelly was bactericidal to the growth of *Shigella*, *Aeromonas* and *Vibrio* spp. at dilutions of 1:14 and *Staphylococcus aureus* at 1:7 dilution. The growth of 2 gram-positive cocci (*Staphylococcus* and *Streptococcus* spp.) and only one gram-negative bacilli (*Shigella* spp.) were totally killed at neat and 1:4 dilutions of bee venom respectively.

Table 2. Bactericidal activity of bee venom and royal jelly on gram-positive and gram-negative organisms

Organisms	Royal jelly (15%)	Bee venom (100 U1%)
1. Pathogenic <i>E. coli</i>	-	-
2. <i>Salm. typhimurium</i>	-	-
3. <i>Salm. enteritidis</i>	-	-
4. <i>Shigella dysenteriae</i>	1:14	1:4
5. <i>Aeromonas hydrophilia</i>	1:14	-
6. <i>Vibrio cholerae</i>	1:14	-
7. Non O1 <i>Vibrio</i>	1:14	-
8. <i>Proteus mirabilis</i>	-	-
9. <i>Staphylococcus aureus</i>	1:7	Neat
10. <i>Streptococcus viridens</i>	-	Neat

DISCUSSION

According to the results obtained from this study, it was noted that the antibacterial effect of bee venom was more pronounced on gram-positive cocci than gram-negative bacilli tested. This fact was in agreement with the findings of Fennell and his colleagues [9]. They reported that more gram-positive organisms (86 per cent) were sensitive to bee venom and to melittin than were gram-negative organisms (46 per cent). Both bee venom and melittin showed no bactericidal effect on *Escherichia coli*, *Salmonella* spp. and *Proteus* spp. They also pointed out that one of the fractions (melittin), which is the largest component (by weight) of bee venom, possesses the antibacterial properties. Although the venom of this study may contain some amount of melittin, it is difficult to compare directly the pure melittin sample with that of the crude, unstandardized venom used in this study. In addition, composi-

tion of bee venom harvested by different method is not necessarily the same.

Being possessing the bactericidal effect especially on gram-positive cocci (Staphylococci and streptococci), it should be considered to apply this venom as a therapeutic agent for the treatment of rheumatoid arthritis, sore throat, upper respiratory tract infections and skin diseases caused by these organisms. This fact has encouraged to make an in-depth studies in order to improve the system of treatment in these diseases. Studies are now underway to determine the fractions of bee venom responsible for different properties like anti-inflammatory, antimalarial and antibacterial actions.

The results also pointed out that royal jelly has a potent bactericidal effect on one gram-positive cocci (Staphylococcus aureus) and gram-negative bacilli (Shigella, Aeromonas and vibrio spp.). Royal jelly, in spite of its higher moisture content, is highly resistant to bacterial, mold or yeast spoilage. Unfortunately, no one can explain the mechanisms of action of this antibacterial effect. Therefore, further investigation to biological activity of royal jelly might be the subject of continual study to draw a constant conclusion after determining the exact components of royal jelly, responsible for the observed bactericidal effect.

Some said that most striking property of royal jelly lies on its ability when use as the food for a developing larvae, to bring about the rather pronounced morphological and physiological differentiation resulting in the formation of a perfect queen. This fact has been

largely responsible for focusing considerable interest on royal jelly in recent years. Researchers and physicians agree that owing to its high vitamins and hormone content, royal jelly must act favourably on the human organs [10]. The results obtained with injectable royal jelly solution administered to the human body particularly in diseased stages were also promising and encouraging. With the discovery of gamma globulin among the components of royal jelly, these results become of a paramount importance for medicine.

As yet only the first step have been made in study of the therapeutic and prophylactic properties of royal jelly and bee venom, further investigations will help to reveal the secrets of these potent remedies produced by bees.

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Occupational exposure to tobacco dust

I. Effects on the respiratory system

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Four hundred and seventy six workers who are exposed to tobacco dust and 112 nonexposed workers from the Cigarette Factory No. (1), Yangon were interviewed for respiratory symptoms and occupational history. General clinical examination was also done and recorded. Anthropometric measurements (height and weight) were taken and ventilatory functions in the form of forced vital capacity (FVC), forced expiratory volume in one second (FEV 1.0), and forced expiratory flow (FEF 25-75%) were tested. Total and respirable dust concentrations were measured at different job areas. The total dust concentration ranged from 3.1 to 12.78 mg/m³ and respirable dust concentration ranged from 1.72 to 5.5 mg/m³ in the production site. Higher prevalence of respiratory symptoms and chronic bronchitis were found in the exposed than the nonexposed workers. Total dust exposure had significant effect on FEV 1.0 ($p < 0.01$) and FEF 25-75% ($p < 0.05$), and respirable dust exposure had significant effect on FEV 1.0 ($p < 0.025$) of females. Mean lung function values of FEV 1.0 and FEF 25-75% of exposed female nonsmokers were significantly lower than the nonexposed ($p < 0.005$ and < 0.05 respectively). Thus in the present study, chronic respiratory effects of occupational exposure to tobacco dust was observed only in females.

INTRODUCTION

Despite the fact that tobacco dust was claimed more than 100 years ago to cause tobaccosis and supposed to effect adversely the respiratory tract [1], only few investigations were carried out on the respiratory effects of occupational exposure to tobacco dust. Respiratory disorders among tobacco workers, in particular dyspnoea, emphysema, and chronic bronchitis [2] and after acute exposure, allergic reactions with a

decrease in ventilatory capacity [3] have been reported. The aim of the present study was to investigate whether there was any chronic effects on the ventilatory functions from occupational exposure to tobacco dust among the workers of the Cigarette Factory No. (1), Yangon.

MATERIALS AND METHODS

A cross-sectional study was carried

out on the workers of the Cigarette Factory No. (1), Yangon, over a period from May 9, 1988 to August 5, 1988. The study population consisted of 615 workers, out of which 503 (82%) workers were from the production site (exposed workers) and 112 (18%) were the clerical staff of the factory (nonexposed workers) with the same socioeconomic status as the exposed workers. They were chosen by the simple random sampling method. Workers of all working ages (18-60 years) were included. In the exposed group, 375 were females and 128 were males, while in the nonexposed group, 44 were females and 68 were males.

Dust measurements were determined by using the Casella Controlled Flow Personal Samples (Casella Landa Ltd.). Both total and respirable dust concentrations were measured for three times in each job section. Mean of the three values was taken for statistical analysis.

Respiratory symptoms, smoking history, and occupational history were recorded using the Modified Medical Research Council Questionnaire [4]. The clinical examination was performed which included an appraisal of the overall health status with special reference to cardiopulmonary system. Those who had history of cardiac disease, history of respiratory disorder before the employment to factory, history of asthma since childhood, recent respiratory tract infection, and chest deformity; those who were pregnant, those who were exposed to other environmental dusts other than tobacco dust and those who had previously worked in the other dusty jobs were rejected.

Height was measured by using the anthropometer to the nearest 0.1 cm

by the Standard Procedure [5].

Ventilatory functions, FVC, FEV 1.0, FEF 25-75% were measured with the Water Sealed Collins Survey Spirometer (Warren E. Collins Inc.). The recording was done with each subject standing without the noseclips. After practice attempts, three satisfactory recordings were taken and the largest FVC and FEV 1.0 were selected [6]. The FEF 25-75% values were calculated from the best curve having the largest sum of FVC and FEV 1.0 [7]. The values were corrected to BTPS before underwent the statistical treatment.

Data analysis

From the study group of 503 exposed workers, 27 subjects were excluded from further statistical analysis, 11 workers on account of the rejection criteria as mentioned previously, 9 subjects for their unsatisfactory spiograms, and 7 subjects from the bale opening and weighing section because this section was not operative during the survey period. All 112 nonexposed workers were included in the analysis. Because of the small sample size ($n = 12$ for both males and females; this number broken down into categories, resulted in sample sizes too small for statistical comparison), exsmokers were excluded from analysis. The nonparametric exact probability test was used for the comparison of the prevalence of respiratory symptoms and chronic bronchitis between the exposed and nonexposed workers. Partial correlation values of each independent variable on the lung function values were calculated using the formula of Spiegel on the IBM computer [8]. Tests of significance were made with the "t" test. Percent of predicted mean lung function values were calculated by using the

predicted equations of Htin Kyaw and co-workers for FVC and FEV 1.0 [9,10] and of Morris et al [11] for FEF 25-75%. Student 't' test was used for comparison between the 2 means. Statistical significance was accepted at the 5% level for each of the tests ($p < 0.05$).

RESULTS

Table (1) shows the mean (range) concentration of total and respirable dusts in the workroom air at the exposed and nonexposed areas. Only the Lamina Conditioning section had total dust concentration that exceeded the threshold limit value (TLV) for inert dust 10 mgm^{-3} .

The prevalence of respiratory symptoms and chronic bronchitis in exposed female nonsmokers were higher than the nonexposed female nonsmokers, although the differences were not significant (Table 2). Prevalance of phlegm in exposed male smokers (17.1%) was significantly higher than the non exposed (2.6%) (Exact probability value = 0.017).

In the exposed male nonsmokers, prevalence of cough, phlegm, and tightness of chest were slightly higher than the non exposed male nonsmokers, but the differences were not significant.

The probability values for test of significance for the influencing variables are reported in table (3). Age had significant effect on all the parameters in both sexes ($p < 0.001$ for all). Height had significant effect on all the parameters in both sexes also ($p < 0.04$, 0.001 , and 0.05 for FVC, FEV 1.0, and FEF 25-75% respectively in females, and $p < 0.001$, 0.001 , and 0.05 correspondingly in males). Regarding the other variables in females, total dust exposure (TDE; mean total dust concentration x years of exposure) had significant effect on FEV 1.0 ($p < 0.01$) and FEF 25-75% ($p < 0.05$); respirable dust exposure (RDE; mean respirable dust exposure x years of exposure) had significant effect on FEV 1.0 ($p < 0.025$) and smoking index (no. of cigarettes/cheroots smoked per day x years of smoking) had signi-

Table 1. Total and resapirable dust concentrations in the workroom air at the job areas

Job Area	No. of Measurements	Mean dust concentration (range),mgm-3	
		Total	Respirable
1. Blending and conditioning (B)	3	4.47(4.20-4.85)	3.10(2.87-3.45)
2. Threshing and separating (A)	3	7.05(6.21-7.46)	4.97(4.44-5.50)
3. Stem conditioning (B)	3	4.34(4.24-4.51)	2.96(2.70-3.44)
4. Lamina conditioning (A)	3	11.33(10.1-12.78)	5.27(5.20-5.41)
5. Spraying (A)	3	7.25(6.90-7.60)	4.20(3.51-4.52)
6. Cigarette making (C)	3	3.80(3.61-3.93)	2.11(2.08-2.32)
7. Cigarette packing and boxing (C)	3	3.33(3.11-3.42)	1.81(1.72-1.94)
8. Nonexposed area	3	1.91(2.01-1.80)	0.70(0.74-0.65)

(A) denotes categorized under Job Area (A) - Heavily Dusty

(B) denotes categorized under Job Area (B) - Moderately Dusty

(C) denotes categorized under Job Area (C) - Lightly Dusty

Table 2. Prevalence of respiratory symptoms and chronic bronchitis in exposed and nonexposed workers

Symptoms	Number of workers (percentage) having symptoms			
	Male		Female	
	Exposed	Nonexposed	Exposed	Nonexposed
Cough				
Nonsmokers	2 (6.9)	0	18 (17.1)	1 (2.3)
Smokers	8 (9.1)	2 (5.1)	26 (27.7)	-
Phlegm				
Nonsmokers	2 (6.9)	0	14 (5.5)	1 (2.3)
Smokers	15 (17.1)	1 (2.6)*	22 (23.4)	-
Tightness of chest				
Nonsmokers	1 (3.5)	0	8 (3.2)	0
Smokers	4 (10.4)	0	19 (20.2)	-
Breathlessness				
Nonsmokers	1 (3.5)	1 (3.5)	17 (6.7)	1 (2.3)
Smokers	3 (3.4)	0	18 (20.0)	-
Chronic bronchitis				
Nonsmokers	0	0	5 (2.0)	0
Smokers	1 (1.1)	0	7 (7.5)	-

*p = 0.017

Table 3. "p" values for tests of significance of each explanatory variable beyond the effects of other variables

Lung Function Tests	Age	Height	Weight	TDE	RDE	Work Duration	Smoking Index
<u>Males</u>							
FVC	0.001	0.001	0.4	0.7	0.9	0.6	0.1
FEV1.0	0.001	0.001	0.3	0.9	0.7	0.6	0.001
FEF25-75%	0.001	0.05	0.5	0.08	0.7	0.5	0.6
<u>Females</u>							
FVC	0.001	0.04	0.4	0.7	0.25	0.6	0.6
FEV1.0	0.001	0.001	0.7	0.01	0.025	0.9	0.2
FEF25-75%	0.001	0.05	0.5	0.05	0.7	0.3	0.02

TDE = Total Dust Exposure

RDE = Respirable Dust Exposure

ficant effect on FEF25-75% ($p < 0.02$). In males, smoking index had significant effect on FEV1.0 ($p < 0.001$) but TDE and RDE had no significant effect on any parameter.

The percent of predicted mean lung function values of female nonsmokers were lower in the exposed than the nonexposed workers, although the differences of FEV1.0 and FEF25-75% were significant only ($t = 3.04$; $p < 0.005$ and $t = 2.01$ and $p < 0.05$ for FEV1.0 and FEF25-75% respectively). Values of female smokers had no counterpart for comparison (table 4). In males of both smoking categories the differences were not significant.

Table 4. Lung function values (MEAN $\pm$ SD) of exposed and nonexposed workers

Lung Function Tests	Exposed	Nonexposed
Males		
Nonsmokers		
	n=29	n=29
FVC	99.8 $\pm$ 10.1	103.0 $\pm$ 9.2
FEV1.0	99.2 $\pm$ 9.8	100.6 $\pm$ 6.1
FEF25-75%	86.3 $\pm$ 20.7	87.1 $\pm$ 10.8
Smokers		
	n=88	n=39
FVC	100.3 $\pm$ 10.8	99.5 $\pm$ 6.3
FEV1.0	98.7 $\pm$ 10.7	99.2 $\pm$ 10.0
FEF25-75%	90.0 $\pm$ 25.2	92.2 $\pm$ 28.1
Females		
Nonsmokers		
	n=253	n=44
FVC	99.9 $\pm$ 11.1	103.5 $\pm$ 14.0
FEV1.0	96.8 $\pm$ 11.0	101.7 $\pm$ 9.7**
FEF25-75%	89.8 $\pm$ 23.0	97.7 $\pm$ 20.1*
Smokers		
	n=94	
FVC	98.8 $\pm$ 14.2	
FEV1.0	92.7 $\pm$ 14.4	
FEF25-75%	77.6 $\pm$ 26.8	

Lung function values are expressed as percent of predicted

* significant difference ($p < 0.05$) between means of exposed and nonexposed workers

**significant difference ($p < 0.005$) between means of exposed and nonexposed workers

DISCUSSION

The effects of tobacco dust on the respiratory tract, in terms of increased prevalence of respiratory symptoms and ventilatory function impairment are not well documented. Only few investigations on the chronic respiratory effects of occupational exposure to tobacco dust have been carried out. In our study, the prevalence of respiratory symptoms and chronic bronchitis was found to be higher in the exposed than nonexposed workers although the only significant difference was found in the prevalence of phlegm in male workers. This might be due either to the effect of dust exposure or the coincidental higher mean smoking index in male exposed than the nonexposed workers. In the study of Valic *et al.* [13], the prevalence of chronic bronchitis and chest tightness was higher in the exposed females than the controls. A significantly higher prevalence of chronic attacks of shortness of breath with wheezing and dyspnoea was found in cigar and cigarette factory workers when compared to a reference population in the study of Veigi *et al.* [14]. Among men there was a relatively constant trend towards an increase in prevalence of respiratory symptoms (cough, phlegm, and wheezing) in the different groups of nonsmokers with short work duration to the smokers with longer work duration. In the present study, the prevalence of respiratory symptoms was compared only between the exposed and nonexposed workers of the same sex and smoking category. A higher prevalence of chronic bronchitis in tobacco workers than in the controls was also seen in the study of Lander and Gravesen [15].

Finding of significantly lower FEV1.0 and FEF25-75% values in the exposed female nonsmokers than the

nonexposed; significant effects of TDE on FEF25-75% of females; significant effect of RDE on FEV1.0 of females in our study indicated that chronic exposure to tobacco dust caused impairment of ventilatory functions in the females tobacco workers. Their mean work duration was 17.1 years and they were working in the cigarette factory where the upper limit of the mean total dust concentration (12.78 mgm/-3) exceeded the TLV for inert dust. This implied that tobacco dust which was generated and existed in the ambient air of the production site went beyond the level where health is endangered. The different effects on ventilatory functions observed in the different sexes may be due to the fact that, although male and female workers were working in the same production section, females worked full time in their working places, while males usually went outside the workroom to perform manual load carrying operations. On account of this, female workers might be inhaling greater amounts of tobacco dust than male workers in the same section and for the same work duration. Ventilatory function impairment was also found in female tobacco workers in the study of Ghosh *et al.* [16]. Similarly, a significant impairment of FEF25-75% in older female tobacco workers of more than 35 years work duration was seen in the study of Veigi *et al.* Lander and Gravesen had also found that cigar factory workers had significantly lower FVC and FEV1.0 values than the controls.

In conclusion, the results of the present study suggest that the chronic inhalation of tobacco dust is harmful to the airways, causing increased prevalence of respiratory symptoms and chronic bronchitis, and ventilatory function impairment. The confirmatory relationship between the causes of ventilatory function

impairment and the effects produced by them in tobacco workers is not known because environmental assessment for the identification and quantification of airborne microorganisms and chemical analysis of tobacco dust could not be done due to the limited resources available.

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Occupational exposure to tobacco dust II. Dose response relationship

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Ventilatory functions, forced vital capacity (FVC), forced expiratory volume in one second (FEV1.0) and forced mid expiratory flow (FEF25-75%) of the 476 workers who were exposed to tobacco dust from the Cigarette Factory No. (1), Yangon were tested. Total and respirable dust concentration were measured at different job areas. The total dust concentration ranged from 3.1 to 12.78 mgm/m³ and respirable dust concentration ranged from 1.72 to 5.5 mgm/m³ in the production site. A significant trend towards a decrease in FEV1.0 values according to increasing dust exposure and work duration ($P < 0.05$) was found among the exposed female workers. Thus in this study, dose-response relationship between the tobacco dust exposure and ventilatory function impairment was observed in female workers.

INTRODUCTION

Although the effects of smoking on health has been studied extensively, the effects of tobacco dust on the respiratory tract are not well documented [1]. The American Conference of Governmental Industrial Hygienists has stated that despite the need to establishing a threshold limit value (TLV), the published documentation is not sufficient [2]. Chronic exposure to tobacco dust caused respiratory function impairment especially in female tobacco workers [3]. In our previous paper

on chronic respiratory effects of occupational exposure to tobacco dust we had found that ventilatory functions of female tobacco workers were significantly lower than the nonexposed and had higher prevalence of chronic respiratory symptoms [4]. The aim of the second part of our study was to investigate whether there is a dose-response relationship between the tobacco dust exposure and ventilatory function impairment.

MATERIALS AND METHODS

A cross sectional study was carried

out on the workers of the Cigarette Factory No. (1), Yangon, over a period from May 9, 1988 to August 5, 1988. A total of 503 exposed workers were studied, out of which 375 were females and the rest 128 were males. Workers of all working ages were included.

For the second part of the study, to determine the dose-response relationship between the dust exposure and ventilatory function impairment, the exposed workers were divided into subgroups according to

- (1) Current job areas
- (2) Total dust exposure (TDE)
- (3) Respirable dust exposure (RDE)
- (4) Work duration (WD)

Measurement of the total and respirable dust concentrations in the different job sections of the exposed area were determined by using the Casella Controlled Flow Personal Samplers (Casella Landa Ltd.).

Criteria for rejection was set up as described in our previous paper [4].

Ventilatory function measurements were made with the Water Sealed Collins Survey Spirometer (Warren E Collins Inc.). Confirmation of the accuracy of equipment was done as described by Gardner *et al.* [5]. Spirometric measurements were taken from 8:00 AM to 11:00 AM. The recording was done according to the method used by Cotes [6] with each subject standing without the nose clips. After practice attempts, three satisfactory recordings were taken and selected the largest FVC and FEV1.0 [7]. The FEF25-75% values were calculated from the single best curve having the largest sum of FVC and FEV1.0 [8]. The values were calculated to BTPS for statistical analysis.

DATA ANALYSIS

From the study group of 503 exposed workers, 27 subjects were excluded from further statistical analysis; 11 workers on account of the rejection criteria as mentioned previously, 9 subjects for their unsatisfactory spiograms and 7 subjects from the bale opening and weighing section because this section was not operative during the survey period. Because of the small sample size ($n = 12$ for both males and females; this number broken down into categories, resulted in sample sizes too small for statistical comparison), exsmokers were excluded from analysis. Comparisons were made for each sex and smoking category separately. Percent of predicted mean lung function values were calculated by using the prediction equation by Htin Kyaw and co-workers for FVC and FEV1.0 [9,10] and of Morris *et al.* [11] for FEF25-75%. Student's 't' test and analysis of variance were used for comparison between and among the different exposure groups, work duration and job areas. Statistical significance was accepted at the 5% level for each of the tests ($p < 0.05$).

RESULTS

Table (1) shows the mean (range) concentration of total and respirable dust in the workroom air of the exposed areas. Mean total dust concentration ranged from 3.1 to 12.78 mgm/m³. Only the Lamina Conditioning Section had total dust concentration that exceeded the TLV for inert dust 10 mgm/m³ [2]. Mean respirable dust concentration were in the range of 1.72 to 5.5 mgm/m³. For further analysis, job areas were categorized into three areas according to the dust concentration

Table 1. Total and respirable dust concentrations in the workroom air at the job areas

Job Area	No. of Measurements	Mean dust concentration (range), mgm^{-3}	
		Total	Respirable
1. Blending and conditioning (B)	3	4.47(4.20- 4.85)	3.10(2.87-3.45)
2. Threshing and separating (A)	3	7.05(6.21- 7.46)	4.97(4.44-5.50)
3. Stem conditioning (B)	3	4.34(4.24- 4.51)	2.96(2.70-3.44)
4. Lamina conditioning (A)	3	11.33(10.01-12.78)	5.27(5.20-5.41)
5. Spraying (A)	3	7.25(6.90- 7.60)	4.20(3.51-4.52)
6. Cigarette making (C)	3	3.80(3.61- 3.93)	2.11(2.08-2.32)
7. Cigarette packing and boxing (C)	3	3.33(3.11- 3.42)	1.81(1.72-1.94)

(A) denotes categorized under Job Area (A) - Heavily Dusty

(B) denotes categorized under Job Area (B) - Moderately Dusty

(C) denotes categorized under Job Area (C) - Lightly Dusty

Table 2. Lung function values (MEAN $\pm$ SD) of exposed workers according to job areas

Lung Function Tests	Job Area (A)	Job Area (B)	Job Area (C)
Males			
Smokers	n = 13	n = 9	n = 66
FVC	104.7 $\pm$ 8.7	103.6 $\pm$ 9.5	99.3 $\pm$ 11.0
FEV1.0	102.1 $\pm$ 8.8	96.4 $\pm$ 10.8	98.3 $\pm$ 11.8
FEF25-75%	92.9 $\pm$ 19.9	75.2 $\pm$ 24.8	91.6 $\pm$ 25.9
Females			
Nonsmokers	n = 110	n = 19	n = 124
FVC	100.6 $\pm$ 11.6	97.3 $\pm$ 9.4	99.6 $\pm$ 11.9
FEV1.0	98.5 $\pm$ 11.1	93.8 $\pm$ 10.1	95.7 $\pm$ 11.9
FEF25-75%	91.1 $\pm$ 21.3	84.6 $\pm$ 21.1	89.4 $\pm$ 24.6
Smokers	n = 22	n = 10	n = 62
FVC	96.7 $\pm$ 14.9	104.4 $\pm$ 14.6	98.6 $\pm$ 13.8
FEV1.0	90.4 $\pm$ 17.3	99.1 $\pm$ 11.7	92.5 $\pm$ 15.5
FEF-25-75%	74.7 $\pm$ 25.8	85.6 $\pm$ 29.8	77.3 $\pm$ 26.0

Lung function values are expressed as percent of predicted

namely, heavily dusty areas (A), moderately area (B) and lightly dusty area (C).

The percent of predicted mean lung function values among the exposed workers according to different job areas are presented in table (2). No significant differences were found

among the mean lung function values of workers of different job areas. In male nonsmokers, values were not shown and analyzed as there were only 2 subjects each in job areas A and B.

In table (3), the percent of predicted mean lung function values among the exposed workers according

Table 3. Lung function values (Mean $\pm$ SD) of exposed workers by work duration

Lung Function Tests	< 10 years	10-25 years	> 25 years
Males			
Nonsmokers	n = 8	n = 14	n = 7
FVC	102.2 $\pm$ 10.4	99.3 $\pm$ 9.8	97.9 $\pm$ 11.7
FEV1.0	105.7 $\pm$ 7.5	96.2 $\pm$ 9.9	97.6 $\pm$ 12.2
FEF25-75%	99.5 $\pm$ 11.3	78.2 $\pm$ 17.3	87.5 $\pm$ 28.4
Smokers	n = 47	n = 29	n = 12
FVC	98.8 $\pm$ 10.1	101.5 $\pm$ 11.6	103.0 $\pm$ 10.6
FEV1.0	98.9 $\pm$ 9.9	96.6 $\pm$ 13.4	103.3 $\pm$ 10.1
FEF25-75%	93.5 $\pm$ 23.9	82.8 $\pm$ 28.2	93.7 $\pm$ 20.3
Females			
Nonsmokers	n = 59	n = 147	n = 47
FVC	101.2 $\pm$ 9.7	100.3 $\pm$ 14.0	97.3 $\pm$ 12.6*
FEV1.0	98.6 $\pm$ 10.5	97.6 $\pm$ 11.3	92.0 $\pm$ 12.4
FEF25-75%	90.1 $\pm$ 20.5	91.3 $\pm$ 22.2	84.7 $\pm$ 27.5
Smokers	n = 9	n = 57	n = 28
FVC	100.9 $\pm$ 14.0	98.1 $\pm$ 15.5	99.5 $\pm$ 11.7
FEV1.0	96.7 $\pm$ 13.6	91.3 $\pm$ 17.3	94.3 $\pm$ 13.2
FEF25-75%	78.6 $\pm$ 21.9	76.5 $\pm$ 27.9	79.5 $\pm$ 23.0

Lung Function values are expressed as per cent of predicted

* significant differences ($p < 0.05$) among the groups with different work duration

Table 4. Lung function values (Mean $\pm$ SD) of exposed workers according to total and respirable dusts exposure (mgm/m³ year)

Lung Function Tests	<80 TDE	>80 TDE	<50 RDE	>50 RDE
Males				
Nonsmokers	n = 20	n = 9	n = 20	n = 9
FVC	100.8 $\pm$ 9.9	97.4 $\pm$ 11.1	101.0 $\pm$ 9.9	97.2 $\pm$ 11.1
FEV1.0	100.1 $\pm$ 9.8	97.0 $\pm$ 11.6	100.4 $\pm$ 10.8	97.2 $\pm$ 11.6
FEF25-75%	87.5 $\pm$ 18.5	83.6 $\pm$ 25.7	87.9 $\pm$ 18.5	83.6 $\pm$ 25.7
Smokers	n = 67	n = 21	n = 70	n = 18
FVC	99.1 $\pm$ 10.0	104.2 $\pm$ 12.2	99.2 $\pm$ 12.7	104.5 $\pm$ 14.7
FEV1.0	98.0 $\pm$ 11.6	100.9 $\pm$ 10.3	97.9 $\pm$ 11.6	101.8 $\pm$ 11.4
FEF25-75%	91.2 $\pm$ 26.2	86.1 $\pm$ 21.6	90.9 $\pm$ 26.7	87.2 $\pm$ 19.0
Females				
Nonsmokers	n = 117	n = 136	n = 120	n = 133
FVC	101.0 $\pm$ 11.4	98.9 $\pm$ 11.7	100.8 $\pm$ 10.8	99.1 $\pm$ 12.2
FEV1.0	98.8 $\pm$ 11.3	95.1 $\pm$ 11.5*	98.3 $\pm$ 11.0	95.4 $\pm$ 11.8*
FEF25-75%	92.4 $\pm$ 21.3	87.6 $\pm$ 23.9	92.1 $\pm$ 21.6	87.7 $\pm$ 23.6
Smokers	n = 33	n = 61	n = 35	n = 59
FVC	95.1 $\pm$ 14.9	100.8 $\pm$ 13.5	95.7 $\pm$ 14.8	100.6 $\pm$ 13.6
FEV1.0	89.9 $\pm$ 17.0	94.2 $\pm$ 14.8	90.7 $\pm$ 14.8	93.9 $\pm$ 15.0
FEF25-75%	73.4 $\pm$ 26.3	79.7 $\pm$ 25.3	74.9 $\pm$ 27.3	79.2 $\pm$ 24.9

Lung function values were expressed as percent of predicted

*significant difference ($p < 0.05$) between means of 2 different exposure groups

to the different TDE and RDE are presented. In males of both smoking categories, mean lung function values of greater exposure group were lower than the lesser exposure group, although the differences were not significant. In female nonsmokers, values of all three parameters were lower in the greater exposure group, but the only significant difference was found in FEV1.0 values ($p < 0.05$). In female smokers, the differences were not significant.

Table (4) shows the percent of predicted mean lung function values of the exposed workers of different work duration. In males of both smoking categories, there was no significant trend towards a decrease in lung function values with increasing WD in any parameter. In female nonsmokers, the only significant trend was found in FEV1.0 values ($p < 0.05$). No significant trend was found for any parameter in female smokers.

DISCUSSION

In our previous paper it had been shown that chronic exposure to tobacco dust caused impairment of ventilatory functions in the female tobacco workers. The determination of the dose-response relationship between the impairment of ventilatory function and tobacco dust exposure was carried out in the present study by comparing the lung function values of the exposed workers in different job areas, dust exposure and work durations.

A possible explanation for not finding any significant difference in ventilatory functions among female workers of different job areas is that those of the lightly dusty area (C) were workers with the most senior service (mean WD = 22.74 ± 6.8), while

those of the highly dusty area (A) had the lowest mean work duration (mean WD = 11.1 ± 5.4). In male smokers, those who worked in the lightly dusty area had the lowest mean work duration and yet no significant difference in ventilatory functions from workers of other job areas was seen. It is probably because the male workers of heavily dusty and moderately dusty areas did not stay in their work site long enough for impairment of pulmonary functions to occur as mentioned in our previous study [4].

Significant trends towards decrease in lung function values with increasing dust exposure and work duration were found in FEV1.0 values of female nonsmokers. Likewise, it was expected that female smokers would have decreasing ventilatory functions with increasing dust exposure and work duration but no significant trend was seen for any ventilatory parameter. This is, however, not altogether surprising when the fact that the subjects tested varied in their smoking habits is taken into consideration.

Based on the above findings in female nonsmokers, it can be inferred that there is a dose response relationship between tobacco dust exposure and ventilatory function impairment in the female tobacco workers.

Very few other studies had been carried out to determine the above dose-response relationship. Although Valic *et al.* [1] found no such relationship, Veigi *et al.* [2] reported a significant impairment of FEF25-75% in male tobacco workers with more than 6 years work duration. A nonsignificant trend towards a decrease in FEV1.0 and FEF25-75% according to increasing work duration was also found in these workers. The older women in their study showed the

lowest functional values.

In conclusion, our data suggests that long term exposure to tobacco dust is harmful to the airways and that the ventilatory function impairment becomes more evident with increasing dust exposure. Our study confirms the need to establishing a TLV for tobacco dust and to performing periodic checks of the environmental and health conditions of these workers.

Further recommendations

Further research that could be extended on this study are

- (1) Environmental assessment for the identification and quantification of airborne microfungi and micro-organisms and chemical analysis of tobacco dust so as to establish a more confirmatory relationship between the causes of respiratory impairment and the effects produced by them in tobacco workers.
- (2) Separate dust measurements for both sexes, to find out if there is a difference in dust exposure between males and females of the same production section, which in the present study was considered a likely cause of the impaired pulmonary function found in females and not in male tobacco workers.
- (3) Longitudinal study of at least 5 years to be able to assess the impairment of ventilatory functions more reliably, which has not been done in the present study due to time limitation.

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Changes in serum aldosterone levels in response to malnutrition and diarrhoea

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The study included 31 children (age 1 to 5 years) who were hospitalized for malnutrition, malnutrition with diarrhoea and acute diarrhoea of less than 48 hrs duration. The controls, consisted of 6 children from the same locality as patients, were apparently healthy and in the same age group. Serum chloride tend to increase in the patients and the increase was more pronounced in the group suffering from both malnutrition and diarrhoea. On the other hand, serum potassium tend to decrease and the decreases were significant in the groups having malnutrition. Serum aldosterone levels were raised in all the three groups of patients namely malnutrition, diarrhoea and malnutrition associated diarrhoea when compared with the control. The increase was still observed even after exclusion of the subjects whose ages lied within the first 1½ year of life for whom the serum aldosterone levels were found to have a sharp rise.

INTRODUCTION

Diarrhoea particularly of young children, is a global health problem, especially in developing countries. Acute childhood diarrhoea stood second in the leading causes of morbidity in 1976, 1977 and 1978 in hospitals of Myanmar [1]. Malnutrition was found to be the most frequent co-existing illness, being observed in about 10% of children with diarrhoea. In these children with diarrhoea and malnutrition, the deaths had not been due to dehydration, but had been due to the co-existing malnutrition. The incidence of hyponatraemia in acute diarrhoea has also been shown to be directly related to the degree of malnutrition [2]. The endocrine homeostatic mechanism may have been responsible for such an incidence and in the present study, the significance of blood aldosterone have been

studied in children with or without malnutrition during diarrhoea and its relationship with the blood electrolyte levels in these patients.

MATERIALS AND METHODS

Subjects

The study was performed on 31 children hospitalized in the pediatric ward of the North Okkalapa General Hospital for malnutrition, malnutrition with diarrhoea and acute diarrhoea of less than 48 hrs duration. The ages of the subjects were between 1 year and 5 years. The subjects were randomly chosen and classified as follows by clinical symptoms and NCHS (National Centre for Health Statistics) standard.

Malnutrition

For the definition of malnutrition,

the cutoff point taken was weight for age being lower than two standard deviation below the NCHS median [3].

Malnutrition with diarrhoea

Malnutrition with diarrhoea cases were assessed by

- (i) acute diarrhoea of less than 48 hrs duration
- (ii) clinical signs of moderately severe dehydration (about 5% body weight less or plasma specific gravity of 1.035 and above) and
- (iii) weight for age lower than two standard deviation below the NCHS median (regarded as malnourished state).

Diarrhoea

Diarrhoea cases were assessed by

- (i) acute diarrhoea of less than 48 hrs duration
- (ii) clinical signs of moderately severe dehydration (about 5% body weight less or plasma specific gravity of 1.035 and above) and
- (iii) weight for age not less than two standard deviation below the NCHS median (regarded as normal nutritional state).

Control

Control group (C) consisted of 6 children with weight for age not less than two standard deviation below the NCHS median who were in the same age group as the patients under study. They were apparently healthy and also living in the North Okkalapa Township.

Sample collection and assays

Blood specimens were collected between 8:00 AM and 9:00 AM after admission to the hospital. Serum aldosterone was assayed according to Radioimmunoassay kit supplied by

Radioassay Systems Laboratories Inc., Immunochem Corp. Serum sodium and potassium were measured by the flame photometer and serum chloride by chloridometer.

RESULTS

Table 1, shows the age, body weight and height of the subjects involved in the present study. Fig. 1 shows the pattern of alterations of the serum electrolytes. The serum sodium tend to rise in the malnutrition with diarrhoea group over the control. Contrary to this, serum potassium tend to decrease only in groups associated with malnutrition. Infact the decreases observed in malnutrition group (M) and malnutrition with diarrhoea (M+D) group were significant, probability levels being less than 0.001 in both cases when compared with the control (C). It may be interesting to note that serum chloride levels ran parallel with serum sodium. Fig. 2 shows the serum aldosterone levels in the four different groups. It was found that serum aldosterone levels were raised significantly in the three disease groups when compared with the control. Based on the study on 135 infants and children, Kowarski *et al.* [4] reported that serum aldosterone level is increased during the first year of life, which could be due mainly to an increased secretion in relation to size [4].

In the present study, it is of interest to note that the sharp rise of serum aldosterone persists up to one and a half year of life (Fig. 3). The reason for the slight discrepancy between present study and the study by Kowarski *et al.* [4] is not yet clear. In order to exclude the possibility that the rise of serum aldosterone found in the disease

Table 1. Description of subjects

Group	Age (Months)		Body weight (kg)		Height (cm)	
	Mean $\pm$ S.E.	Range	Mean $\pm$ S.E.	Range	Mean $\pm$ S.E.	Range
1. Control (C) (n = 6)	23 $\pm$ 2	17-36	13.92 $\pm$ 0.66	12-15	89.5 $\pm$ 2.5	82-100
2. Malnutrition (M) (n = 14)	25 $\pm$ 2	15-36	7.30 $\pm$ 0.44	3.6-10	76.4 $\pm$ 1.2	68-82
3. Malnutrition with Diarrhoea (M + D) (n = 7)	18 $\pm$ 2	13-24	6.18 $\pm$ 0.65	4.0-8.6	73.2 $\pm$ 2.4	65-83
4. Diarrhoea (D) (n = 10)	20 $\pm$ 2	16-30	13.00 $\pm$ 0.52	10.5-13.5	85.0 $\pm$ 1.5	78-92

n = number of subjects
S.E. = Standard Error

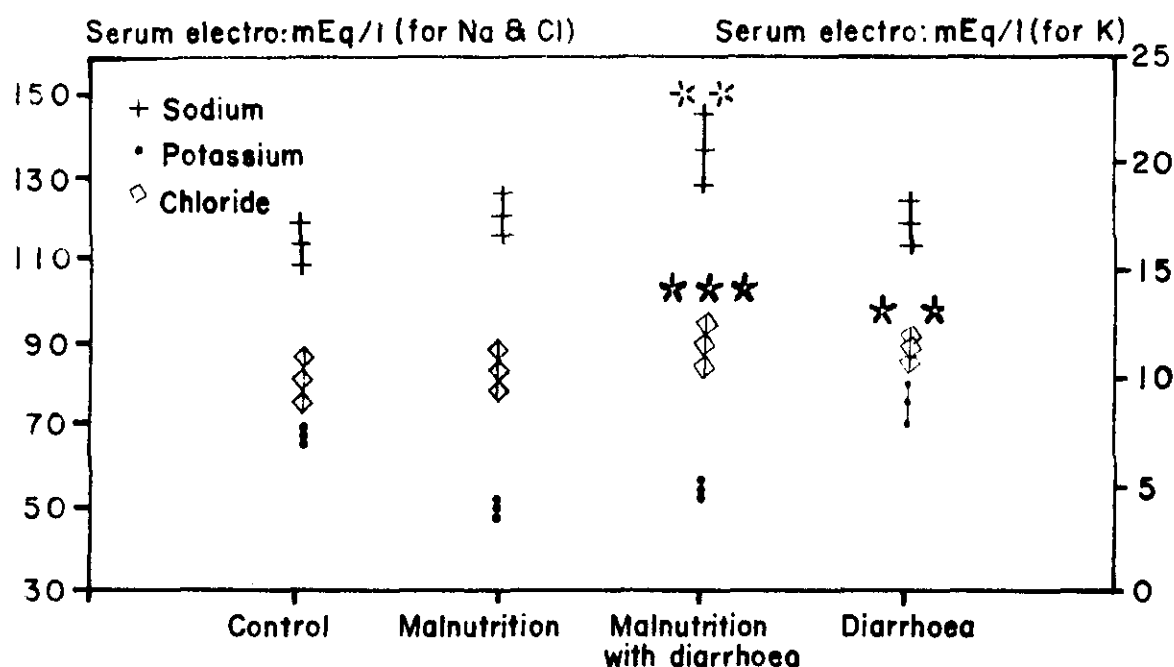


Fig. 1. Serum electrolytes in different groups of subjects; **p<0.05; ***p<0.01

groups, could be the result of inclusion of subjects who had ages up to 1½ yr, those having ages up to 1½ yr (or 18 months) were excluded from the four groups and the serum aldosterone levels were again compared. The rises in serum

aldosterone were still observed in the disease groups, namely malnutrition (M), malnutrition with diarrhoea (M+D) and the diarrhoea (D) groups when compared with the control as shown in Fig. 4.

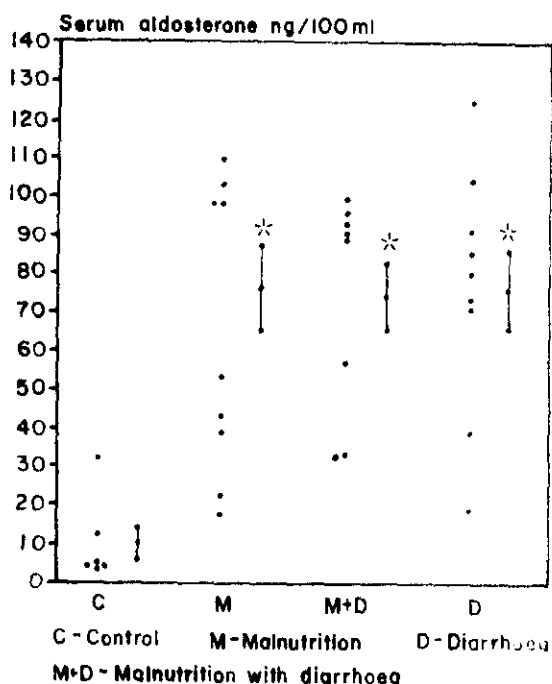


Fig. 2. Serum aldosterone levels in different groups of subjects * $p < 0.001$

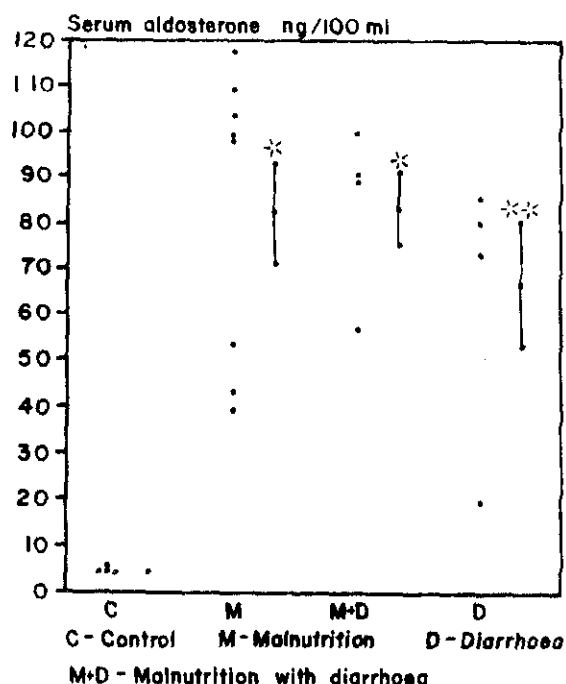


Fig. 4. Serum aldosterone levels in different groups of subjects excluding those under 18 months of age * $p < 0.001$; ** $p < 0.01$

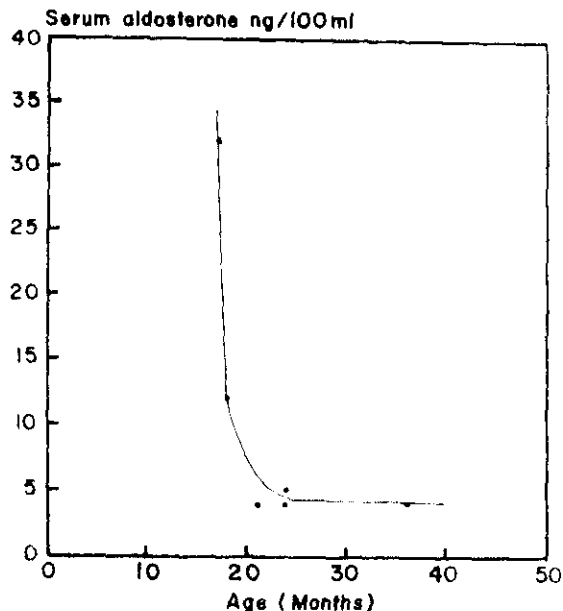


Fig. 3. Correlation of serum aldosterone concentration with age in control (normal) subjects

DISCUSSION

The interaction between malnutrition and diarrhoeal diseases is believed to be mutually postulated causative. A bidirectional relationship is postulated where in diarrhoea precipitates and exacerbates malnutrition and malnutrition predisposes to diarrhoea [5]. Of these two interactions the effect of diarrhoea in malnutrition appears to be documented, since diarrhoea has been reported to exert a significant negative impact on child growth in Guatemala [6] and in Gambia [7]. Our results seem to be consistent with the above speculation, since lowest body weight and height were observed in the group of malnutrition with diarrhoea (M+D). However, it should be noted that the mean age of the

subjects in the malnutrition with diarrhoea group happened to be lowest in the present study and the impact of this on growth should not also be overlooked.

Serum sodium level may be an important indicator for the study of sodium homeostatic imbalance. Moreover, Samadi *et al.* [2] have found that the incidence of hyponatraemia in acute diarrhoea has been shown to be directly related to the degree of malnutrition. Thus, serum sodium (serum potassium and chloride, in addition) were measured in the present study. However, only apparent hypernatraemia was observed in the group of malnutrition (M) and diarrhoea (D) in contrast to a significant hypernatraemia found in the malnutrition associated diarrhoea group (M+D). It may be of interest to note that serum chloride ran parallel with serum sodium, the reason for which may lie in that Na^+ and Cl^- are chief extracellular cation and anion and their metabolism is regulated generally in an analogous manner under the action of aldosterone, a mineralocorticoid secreted by adrenal cortex. The aldosterone may govern water and electrolyte metabolism [8] and hence its levels were measured in the present study. Serum aldosterone levels were raised in all the disease groups when compared with the control. Although the exact mechanism by which the serum aldosterone levels were raised is not yet clear, it appears that the increase of serum aldosterone concentration may reflect increased aldosterone secretion from the adrenal cortex and decreased metabolic clearance of aldosterone.

It has been reported that serum aldosterone level could be increased during the first year of life probably due to increased secretion

in relation to size [4]. Since the present study dealt with children, it may be appropriate to look at the pattern of aldosterone levels with age. We found a similar rise of aldosterone concentration (Fig. 3), but this rise persists up to 1½ yr (or 18 months) apparently slightly different from the finding of Kowarski *et al.* [4], who found that the rise was confined only to first year of life. The aldosterone levels of these subjects were excluded and the remaining subjects were again compared. The increase in serum aldosterone levels was still observed in all the disease groups as found earlier. Although the clinical significance for such an incidence of increased serum aldosterone level is not yet clear, the involvement of endocrine homeostatic mechanism could be suggested in the diseases under study.

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Botanical identification and chemical investigation of

KYET-THAHIN

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KYET-THAHIN an indigenous Myanmar medicinal plant, whose leaves is being claimed by local people to be effective in jaundice, was identified to be Sauropus albicans Blume. Phytochemically, it consists of flavonoid compound as a major chemical constituents.

INTRODUCTION

An indigenous medicinal plant KYET-THAHIN growing wild in Kyaik-kamaw area in Tanyin, Yangyi-aung area in Twante, Gyobu, Taikkyi and Yangon has been claimed as a good remedy for jaundice among local people. This plant is not only use for medicinal purposes but it is also cultivated in house garden for its edible value.

According to Hundley, 1987 [1] and San Khin and Tha Myat, 1970 [2] KYET-THAHIN was identified as Phyllanthus niruri Linn., belonging to the family Euphorbiaceae. Khine Khine Nyunt, 1987 [3] has identified Phyllanthus niruri as TAUNG-ZEE-BYU.

The plant Phyllanthus niruri Linn., has been claimed to be effective in the treatment of infective hepatitis [4,5]. Presence of two alkaloids 4-methoxy securinine and 4-methoxy-non-securinine have been reported from this plant [6]. In addition to the alkaloids, a substance responsible for the inactivation of hepatitis B surface antigen (HBsAg) in-vitro has also been reported from this plant [7].

Since these two plants, KYET-THAHIN

and Phyllanthus niruri has been claimed for the same medicinal value, it is of our interest to determine its true botanical identity of KYET-THAHIN and to detect its chemical constituents and also to clarify the confusion of one Myanmar name KYET-THAHIN given to two different plants.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study was collected during the rainy season (July to October) from Yangon area, and was identified by the Botany section, Pharmacology Research Division, Department of Medical Research (DMR), Voucher specimen HDM No. 102 is deposited in the herbarium of DMR.

Preparation of plant extract

The air dried leaves was coarsely powdered in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, USA). The powdered material (10 gm) was extracted with 95% ethanol in a Soxhlet extractor for 30 min. The extract was evaporated in a vacuum evaporator (Rotavapor, RE-121, BUCHI

Phytochemical test

The ethanolic extract of KYET-THAHIN was tested for the presence of alkaloids with Dragendoff's reagent, flavonoids with magnesium turning and concentrated sulphuric acid, glycosides by Fehling test, phenolic compounds by ferric alum test and saponins by frothing test.

Extraction method of flavonoids

The extraction of flavonoid aglycones in hydrolysed leaf extract, was conducted according to the procedure published in *Phytochemical Methods* [13]. About 10 gm of plant material was hydrolysed with 2M HCl in Soxhlet for 30 min. at 100°C. The cooled extract was then filtered and partitioned with ethyl acetate. The ethyl acetate fraction was concentrated to dryness and directly used for TLC studies.

Isolation of quercetin and kaempferol

The ethyl acetate fraction of leaf extract was dissolved in methanol and aliquots chromatographed one dimensionally, along side authentic markers, in three solvents: Forestal (acetic acid-Conc. HCl-water, 30:3:10), 50% HOAc (50% aqueous acetic acid), and BAW (m-butanol-acetic acid-water, 4:1:5, top layer). Luteolin, apiginin, myrecetin, quercetin and kaempferol were used as authentic markers and cellulose plates (Schleicher & Schuell) were used, and chromatograms were viewed under UV light (Camag, UV-Cabinet II). Two distinct yellow fractions correspond to authentic quercetin and kaempferol were isolated by preparative TLC using the same plates and used for co-tlc study and UV spectral data measurement.

KYET-THAHIN a perennial undershrub with erect slender stem, basal branches, simple alternate, pinnate, ovate-lanceolate leaves with axillary clustered inflorescence, monoecious, greenish-red, 1 to 2 obovate rounded, pistillate flowers in the upper axils and 2 to 3 disciform staminate flowers in lower leaf axil. Fruit of globose berries of white colour tinged with pink colour. These characters are in conformity as mentioned in Hooker (1885), Brandis (1971), Yin Htay (1976) and Tint Naing (1976) and Khin Htwe (1984) [8,9,10,11,12]. From these taxonomic characters, presented in Plate 1 and 2 this plant was confirmed to be Sauropus albicans Blume. belonging to the family Euphorbiaceae.

To clarify the confusion of one Myanmar name KYET-THAHIN given to two different species belonging to the same family, the distinguishing features are presented for comparison in Table 1. The morphological characters of Phyllanthus niruri Linn. presented (Plate 3 & 4) are cited from Khine Khine Nyunt, 1987 [3].

The results of the phytochemical tests on Sauropus albicans Blume. are presented in table 2. The tests were positive for alkaloid, flavonoid, glycoside and phenolic compounds.

Preliminary qualitative test has shown the significant presence of flavonoids and thus flavonoid aglycone was isolated from the hydrolysed plant extracts. R_f values in different solvents and colour characteristics with and without ammonia of two fractions of plant extract and authentic quercetin and kaempferol were found to be similar (Table 3). Co-tlc study and UV spectral data of isolated fractions

Table 1. Distinguishing features of Sauropus albicans Blume. and Phyllanthus niruri Linn.

Morphological characters	<u>Sauropus albicans</u> Blume.	<u>Phyllanthus niruri</u> Linn.
Habit	Perennial undershrub	Annual or perennial herb.
Stem	Woody	Herbaceous
Stipule	Deltoid-lanceolate	Lanceolate
Leaf	Alternate, 4.7-5.8cm x 2.0-2.6cm, ovate-lanceolate, tip acute, base obtuse	Alternate, 4.5-10.0mm x 2.2-4.0mm, linear-oblong, tip rounded, base obtuse
Flower	Monococious, tri-merous apetalous	Monococious, penta-merous apetalous
Staminate (male) flower	Greenish-red, 2 to 3 flowered in the lower leaf axil of the flowering branch	Greenish-white, mostly 2, sometimes 3, in the upper portion of the flowering branch
Sepals	(6) united, in two series, disciform, 6 lobed with thickened rim, mouth very small with superficial thickening that meet around the stamen	5, free, imbricate, ovate
Stamen	(3), filament united in a very short fleshy truncate, 3-armed, 3-gonous column; anther sessile, attach to the ends of the angles of the column arms; disk absent.	3, connate at the base, staminal tube very short, disk glandular, lobed.
Pistillate (female) flower	Greenish-red, 1 to 2 flowered in the upper leaf axil of the flowering branch	Whitish-green, mostly solitary, sometimes 2, usually in the lower portion of the flowering branch
Sepal	(6) united, in two series, obovate-rounded	5, free, oblong
Ovary	Globose, style 3, connate, depressed spreading, stigma trifid, radiate with 3-incurved arms; disk absent	Globose, style 3-fid, very short, stigma simple; disk present, annular, lobed
Fruit	Berry, globose, white tinged with pink colour	Loculicidal capsule, depressed, globose, smooth, thinly crustaceous, explosive, densely warty
Seed	Ovoid	Echinate, ear-shaped

Table 2. Chemical constituents of the ethanolic leaf extract

Compound	Degree of presence
1. Alkaloid	+
2. Flavonoid	+++
3. Glycoside	++
4. Phenolic compounds	++
5. Sugar	-
+++ appreciable quantity ++ moderate quantity	
+ trace - absent	

Table 3. R_f values and spectral data of isolated compounds and authentic markers

Sample	R _f values (x100)			UV absorption maxima (nm)
	Forestal	50% HOAc	BAW	
Isolated				
fraction 1	40	36	64	255, 374
quercetin	41	36	64	255, 374
Isolated				
fraction 2	54	40	83	267, 368
kaempferol	55	39	83	368, 368

HABIT



PLATE 1

FLORAL PARTS.

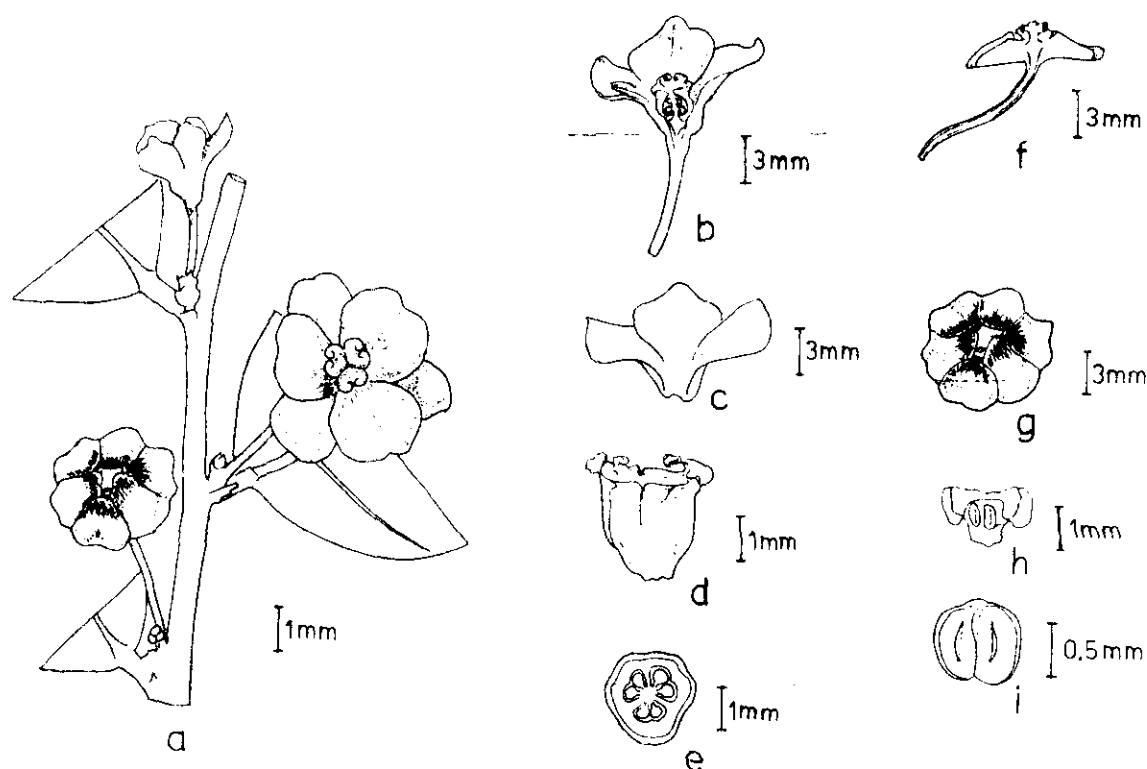


PLATE 2

of the hydrolysed plant extract confirmed those as quercetin and kaempferol (Table 3).

The experimental findings revealed that KYET-THAHIN is Sauropus albicans Blume. and not Phyllanthus niruri Linn. As reported by Khine Khine Nyunt, 1987 [3], appreciable quantity of alkaloid was detected in the leaves of Phyllanthus niruri Linn;.

Flavonoid compound as a major chemical constituents was isolated from Sauropus albicans Blume. leaves in the present study. The differences in the chemical constituents between these two plants was also noted. Therefore, plants should not be accepted with Myanmar name only, without proper botanical identification and chemical investigation, so that there will be less confusion for medicinal plant research.

HABIT



PLATE 3

FLORAL PARTS

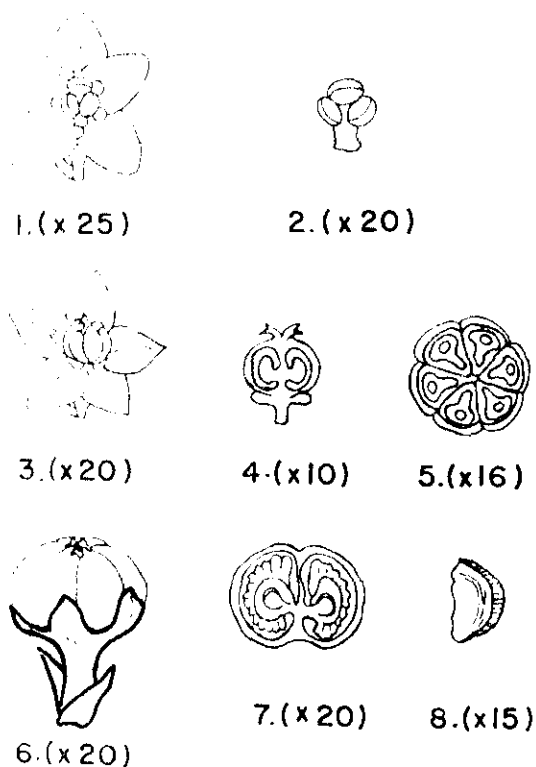


PLATE 4

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Pharmacognostical identification of market Pwaygaing leaf

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Pwaygaing leaflets sold in the market for its medicinal value as purgative and laxative drug and also as one of the ingredients in many of the traditional medicine formulations (TMFs) was pharmacognostically identified to be Cassia angustifolia Vahl.

INTRODUCTION

Pwaygaing leaflets are sold in the market for its purgative and laxative value. It is also known to researchers as Senna. This plant is widely distributed and also cultivated in medicinal herbal gardens throughout Myanmar.

Pwaygaing leaflets are used in certain traditional medicine formulations (TMFs) as a major ingredient in TMFs 09, 10 and 12. The TMFs are manufactured at the production units at the Department of Traditional Medicine, Yangon. The crude drug ingredients used by the manufacturers are procured from the market. They are never collected by the manufacturers nor scientifically identified and authenticated. Since own collection is not performed, there is no assurance of its correct specific name for Pwaygaing.

According to Hundley and Chit Ko Ko [1] and San Khin [2], two species of Cassia namely, Cassia acutifolia Delile., and Cassia angustifolia Vahl. are mentioned for Pwaygaing, whereas Hundley [3] reported only one specie Cassia angustifolia Vahl.

To clarify the above information, the only possible method of identification is by applying the established

pharmacognostic technique using the quantitative microscopy method employed by Trease and Evans [4].

The present investigation is conducted to confirm the correct specific name of Pwaygaing used individually in traditional medicine, as well as in the TMFs.

MATERIALS AND METHODS

Materials

Air dried Pwaygaing leaflets undergoing investigation was supplied by the production unit from the Department of Traditional Medicine, Yangon, together with the TMF-09, TMF-10 and TMF-12.

Method

1. Identification of Pwaygaing leaflets
 - 1.1. Macroscopical study was conducted for its morphological characters from descriptions given in Trease and Evans [4], Wallis [5] and Youngken [6].
 - 1.2. Quantitative Microscopy
For quantitative studies, specimens were cleared in a solution of chloral hydrate on a boiling water bath, stomatal index, vein-islet

and vein-termination numbers were determined according to Trease and Evans [4].

RESULTS

1. Macroscopical characters

Leaves are yellowish-green, measuring 3.0 to 4.8 cm in length and 0.6 to 1.4 cm in width. It is inequilaterally lanceolate with broadly acute apex and less conspicuously asymmetric base, puberulous with warty trichomes on both surfaces. (Table 1 and Fig. 1c)

2. Microscopical characters

Powder is dull greenish yellow colour with characteristic odour and mucilaginous bitterish, unpleasant taste. Many rubiaceous or paracytic (2-parallel subsidiary cell) and occasionally ranunculaceous or anomocytic (3 irregular subsidiary

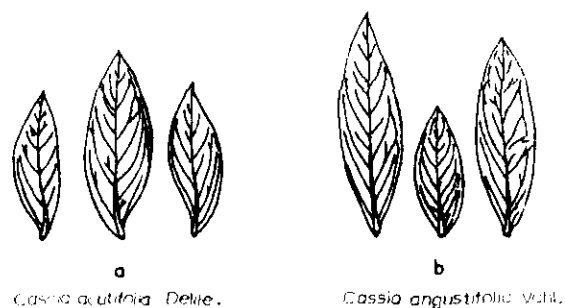


Fig. 1. Three types of leaflets
a. *Cassia acutifolia* Delile.
b. *Cassia angustifolia* Vahl.
c. Pwaygaing market sample

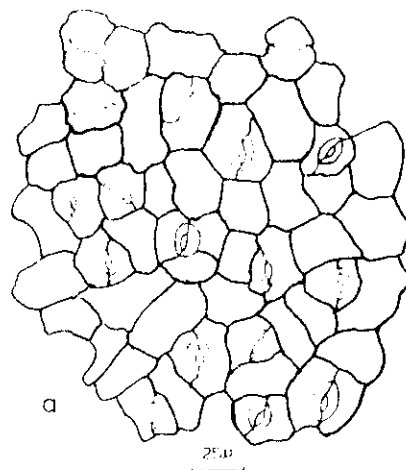
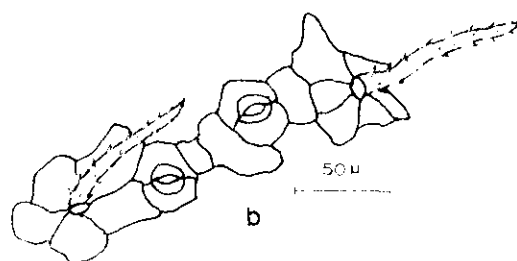


Fig. 2. a. Two types of stomata, Paracytic (Parallel-celled), Anomocytic (three irregular-celled).

b. Distance between each trichome

celled) stomata are present on both surfaces in the ratio of about seven to one (Fig. 2a). Average distances between each trichome is about six epidermal cells (Fig. 2b).

3. Quantitative values

The results of the quantitative values of stomatal index, vein-islet and vein-termination are presented in table 2.

Table 1. Differences in macroscopical characters between Cassia acutifolia Delile., and Pwaygaing

No.	Specis	Colour	Shape	Lamina	Size
1.	<u>Cassia acutifolia</u> Delile. Alexandrian Senna	Greyish-green	Inequilaterally lanceolate or lance-ovate, acute or mucronate apex, conspicuously more asymmetric at base, rather more broken and curled at edges		2.0 to 2.5 cm, rarely exceed 4.0 cm in length and 0.6 to 1.0 cm in width
2.	<u>Cassia angustifolia</u> Vahl. Indian or Tinevelley Senna	Yellowish-green	Inequilaterally lanceolate, broadly acute apex, less conspicuously asymmetric at base, seldom broken and usually flat owing to compression		2.0 to 5.0 cm, seldom exceed 5.0 cm in length and 0.6 to 1.5 cm in width
3.	Myanmar Market Pwaygaing leaflets	Yellowish-green	Inequilaterally lanceolate, broadly acute apex, less conspicuously asymmetric at base, not broken and usually flat		3.0 to 4.8 cm in length does not exceed 5.0 cm and 0.6 to 1.4 cm in width

DISCUSSION

Macroscopical, microscopical characters and quantitative values of market Pwaygaing leaflets are presented in table 1 and 2, together with the characters of Cassia acutifolia Delile., and Cassia angustifolia Vahl. cited from Trease and Evans [4], Wallis [5] and Youngken [6].

Table 1 and Fig. 1 represent the macroscopical character differences between Cassia acutifolia Delile., and Pwaygaing market sample. It also reveals that the characters of Cassia angustifolia Vahl., are in agreement with Pwaygaing.

From table 2, it was observed that the powder colour, the range and average quantitative values of stomatal index of both upper and lower epidermi surfaces; vein-islet and vein-termination number; stomata type and ratio, and the average distance between each hair are in conformity with Cassia angustifolia Vahl.

Therefore the findings from this study reveals the correct specific name for Pwaygaing to be Cassia angustifolia Vahl., which supports to Hundley [3], that there is only one specie of Senna, grown in Myanmar, which majority of the people know as Pwaygaing.

Table 2. Differences in microscopical characters between Cassia acutifolia Delile., and Pwaygaing

Species	Powder colour	Average distance between each trichome	Stomata type and ratio	Stomatal Index		Vein-islet number	Vein-termination number
				Upper	Lower		
1. <u>Cassia acutifolia</u> Delile. Alexandrian Senna	Light green	Hairs more numerous; the average distance about three epidermal cells	Most of the stomata have two subsidiary cells only	11.4- <u>12.4</u> -13.3	10.8- <u>11.8</u> -12.6	25.0- <u>26.0</u> -29.5	33.0 - 40.0
2. <u>Cassia angustifolia</u> Vahl. Indian or Tinevelley Senna	Dusky greenish yellow to light olive	Hairs less numerous, the average distance about six epidermal cells	The stomata having two or three subsidiary cells respectively are in the ratio of about 7 to 3	17.1- <u>19.0</u> -20.7	17.0- <u>18.3</u> -19.3	19.5- <u>21.0</u> -22.5	26.0 - 33.0
3. Myanmar market Pwaygaing	Dull green	Hairs less numerous, the average distance about six epidermal cell	The stomata having two or three subsidiary cells respectively are in the ratio of about 7 to 1	17.0- <u>19.0</u> -21.15	16.0- <u>18.1</u> -19.6	15.0- <u>22.1</u> -23.0	22.0 - 33.0

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letter to the editor

Monitoring the discharge of leprosy cases using mice inoculation test

During 1975, that was during the days of Dapsone monotherapy using mouse inoculation test we were able to prove that there were leprosy patients who are resistant to Dapsone. At the time 30 skin biopsy specimens from lepromatous leprosy patients were inoculated into foot pad of mice. Of these 15 cases showed high grade resistance and 3 untreated cases showed primary resistance to Dapsone [1].

WHO introduced multidrug therapy in 1982. Over the past 5 years, it has cured over 2 million of the World known leprosy patients in 91 countries [2].

But there are reported cases who showed resistance to rifampicin after multidrug therapy. Hence in Myanmar it may be needed to follow some of the doubtful resistant cases with animal model.

Ofloxacin was introduced in 1985 by the Japanese for the treatment of infection, particularly of the urinary and respiratory tracts.

Ofloxacin also kills the leprosy bacillus by inhibiting an enzyme that controls the way DNA coils itself inside the bacillus. In 1986 French Researcher Jacques Grosset of the Pitie-Salpetriere Hospital Medical School in Paris found Ofloxacin to be second only to rifampicin in the speed and efficacy with which it killed leprosy bacillus in laboratory animals [2].

The trials of the new ofloxacin-rifampicin treatment are now organised by TDR AND WHO's leprosy unit.

It will take place in seven countries including Myanmar comparing the new regimen with standard multidrug therapy. Results of trials which will involve over 4000 patients will be available in four or five years.

For such trials, animal model may be needed to follow some of the patients who may show doubtful resistance to the drugs. This is to let fellow scientists know that DMR still has the technology of monitoring drug resistance among leprosy patients using mice inoculation test.

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