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**Association of chronic complications of diabetes mellitus and
presence of risk factors**

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Chronic complications of diabetes are known to be associated with certain risk factors. Once complications develop, they will never regress, denoting that reduction of these factors as much as possible, is the best way to deter the complications. To determine such risk factors, a study was done on diabetic patients with a sample size of 315 from Diabetic Clinic and Wards 1+2, Yangon General Hospital. There were 281 NIDDM and 341 IDDM patients. The study on NIDDM patients shows that physical inactivity and age of the patients were associated with the development of retinopathy. The following factors were identified as being associated: hypertension, high serum cholesterol and age of the patient. Smoking is also associated with neuropathy. The possible risk factors for nephropathy were age of patient, hypertension, alcohol and hyperuricaemia. Hypertension, age of the patient, male sex and physical inactivity were found to be important risk factors for CVA. For IHD, contraceptive pills, physical inactivity and age of patients were possible risk factors. On the other hand, physical inactivity, hypertension, age of patient and duration of disease were important for peripheral vascular disease. Our study spotlighted that age factor is the mainstay in the development of chronic complications in both NIDDM and IDDM. In NIDDM physical inactivity predisposes to retinopathy and atherosclerotic complications. Hypertension enhances neuropathy, nephropathy, CVA and peripheral vascular disease. However, in IDDM, the duration of diabetes and fasting blood sugar levels are significant risks in the development of retinopathy and nephropathy.

INTRODUCTION

Diabetes mellitus is a disease of metabolic dysregulation of glucose, accompanied by characteristic long term complications. The complications that are specific to diabetes include microvascular complications like retinopathy, nephropathy and neuropathy. Diabetes is also accompanied by substantial increase in macrovascular atherosclerotic disease of large vessels, including cardiac, cerebral and peripheral vascular disease. Progression of these complications represents a major threat to the

mortality and morbidity of diabetes, with loss of life, limb or vision. New possibilities have appeared for limiting the progression of these complications. Evidence suggest that improved control of the diabetic state and alteration of risk factors will reduce the complications.

Effectiveness of methods of prevention and treatment of complications depends upon their timely application. Therefore it is crucial for early identification of risk factors and removal or modification of these if possible.

The aim of this study was to analyse several clinical and biochemical parameters,

1. to identify those subsets of patients likely to develop these complications
2. to elucidate whether the incidence of diabetic complications were related to any of twelve variables (risks).

MATERIALS AND METHODS

A total of 315 diabetic patients of the diabetic clinic and in-patients of wards 1 & 2, Yangon General Hospital were included in this study over a three year period starting from first January 1991. A complete history and physical examination including neurological examination and dilated ophthalmoscopy was performed. Laboratory workup included blood sugar estimation, serum cholesterol, urea, uric acid, urinalysis, ECG, and CXR. Patients with chronic complications were correlated with twelve clinical and laboratory variables. Data were analysed statistically using the Chi square test, Student's 't' test and Odds ratio.

RESULTS

1. Age and Sex Distribution: in NIDDM, the female male ratio was 1.9:1. In IDDM, the male female ratio was 1.6:1. In NIDDM, mean age of males was 59.34 years and females 54.72 yr. In IDDM, mean age of males was 27.85 years and females 28.3 years.
2. Mean weight in pounds in NIDDM: was 125.7 lbs (95% CI 123.31-128.09) accounting for 30.8% being obese.
3. Duration of diabetes in years: In NIDDM ranged from 1-20 years with mean $4.44 \pm \text{SE}0.30$. In IDDM ranged from 1-4 years

with mean $2.7 \pm \text{SE}0.41$.

4. Age of onset: In NIDDM ranged from 30 to 78 years with mean $48.66 \pm \text{SE}0.66$. In IDDM ranged from 13 to 37 years with mean $25.25 \pm \text{SE}1.80$.
5. A family history of diabetes: In one or both parents was found in 20.64% of NIDDM and 23.58% of IDDM, overall in 20.9% of patients.
6. Race: 69.2% were Myanmar, 22.85% were Indians and 4.18% were Chinese.
7. Complications (Table 1): 72.95% of NIDDM and 35.29% of IDDM had complications. Among patients with NIDDM, commonest complications were hypertension 46.62%, neuropathy 33.45%, ischaemic heart disease 28.83% and retinopathy 20.28% and nephropathy 19.93%. Among patients with IDDM, the commonest complications were neuropathy 14.71%, retinopathy 11.76%, nephropathy and hypertension 8.82%.
8. Risks: In NIDDM, significant risks for RETINOPATHY were physical inactivity (Odd Ratio 4.25) and age of patient ($p < 0.025$) (Fig. 1). In NIDDM, significant risks for NEUROPATHY were hypertension (OR 2.20), hypercholesterolaemia (OR 4.41), smoking (OR 1.96) and age ($p < 0.001$) (Fig. 2). In NIDDM, risk factors for NEPHROPATHY were hypertension (OR 3.32), alcohol (OR 2.18), hyperuricaemia (OR 2.78) and age ($p < 0.001$) (Fig. 3). Significant risk factors for atherosclerotic complications. Risks for cerebrovascular accidents were hypertension (OR 3.58), physical inactivity (OR 4.08), age ($p < 0.01$) and male sex (OR 2.47) (Fig. 4). Risks for peripheral vascular disease were hypertension (OR 2.41), physical inactivity (OR 4.56) and age ($p < 0.01$).

Table 1. Percentage distribution of chronic complications in diabetes

	NIDDM (281)				IDDM (34)			
	Male	Female	Total	%	Male	Female	Total	%
Retinopathy	18	39	57	20.28	3	1	4	11.76
Background	16	33	49	17.44	3	1	4	11.76
Proliferative	2	4	5	1.78	0	0	0	0.00
Advanced	1	2	3	1.07	0	0	0	0.00
Atherosclerosis	58	107	165	58.72	4	2	6	17.65
Hypertension	45	86	131	46.62	2	1	3	8.82
I.H.D.	25	56	81	28.83	0	1	1	2.94
P.V.D.	5	13	18	6.41	0	1	1	2.94
C.V.A.	13	10	23	8.19	0	0	0	0.00
Neuropathy	36	58	94	33.45	3	2	5	14.71
Nephropathy	24	32	56	19.93	0	3	3	8.82
Anyone or more	70	135	205	72.95	8	4	12	35.29

I.H.D. = Ischaemic heart disease

P.V.D. = Peripheral vascular disease

C.V.A. = Cerebrovascular accident

Risk for ischaemic heart disease were OC pills (OR 1.12), physical inactivity (OR 7.08) and age ($p < 0.01$).

Significant risk factors for complications in IDDM (Fig. 5). Risks for NEPHROPATHY were duration of diabetes ($p < 0.05$), age ($p < 0.05$) and fasting blood sugar levels ($p < 0.001$). Risks for RETINOPATHY were family history (OR 4.0), smoking (OR 2.75) alcohol (OR 5.0), age ($p < 0.001$), duration of diabetes ($p < 0.05$) and fasting blood sugar ($p < 0.005$).

DISCUSSION

There was predominance of NIDDM 89.2% in our study. This is comparable to a study in primary health care setting by Lee in Singapore where 91.5% were NIDDM [1]. In an Audit of diabetes in General Practice in Bristol by Kemple 75.8% were NIDDM [2].

In NIDDM patients the female male ratio was 1.9:1 comparable to Sudan study by Elmahdi with identical ratio [3]. Mean age of NIDDM was 57.03

years while mean age of IDDM was 28.3 years. 30.8% of NIDDM were obese.

Duration of diabetes in NIDDM ranged from 1 to 20 years while in IDDM ranged from 1 to 4 years.

A family history of diabetes was present in 20.9%, 69.2% of patients were Myanmar.

72.95% of NIDDM and 35.29% of IDDM had complications. In NIDDM the commonest complications were hypertension 46.62% neuropathy 33.45%, ischaemic heart disease 28.83%, retinopathy 20.28% and nephropathy 19.93%. In primary health care setting in Singapore by Lee [1], hypertension was found in 38% of diabetics. Comparable results were found in Sudan study by Elmahdi [3] where commonest complication was neuropathy 31.5% followed by retinopathy 17.4%.

Significant risks for retinopathy in NIDDM in our study was increasing age and physical inactivity. Chen reported that risk of retinopathy

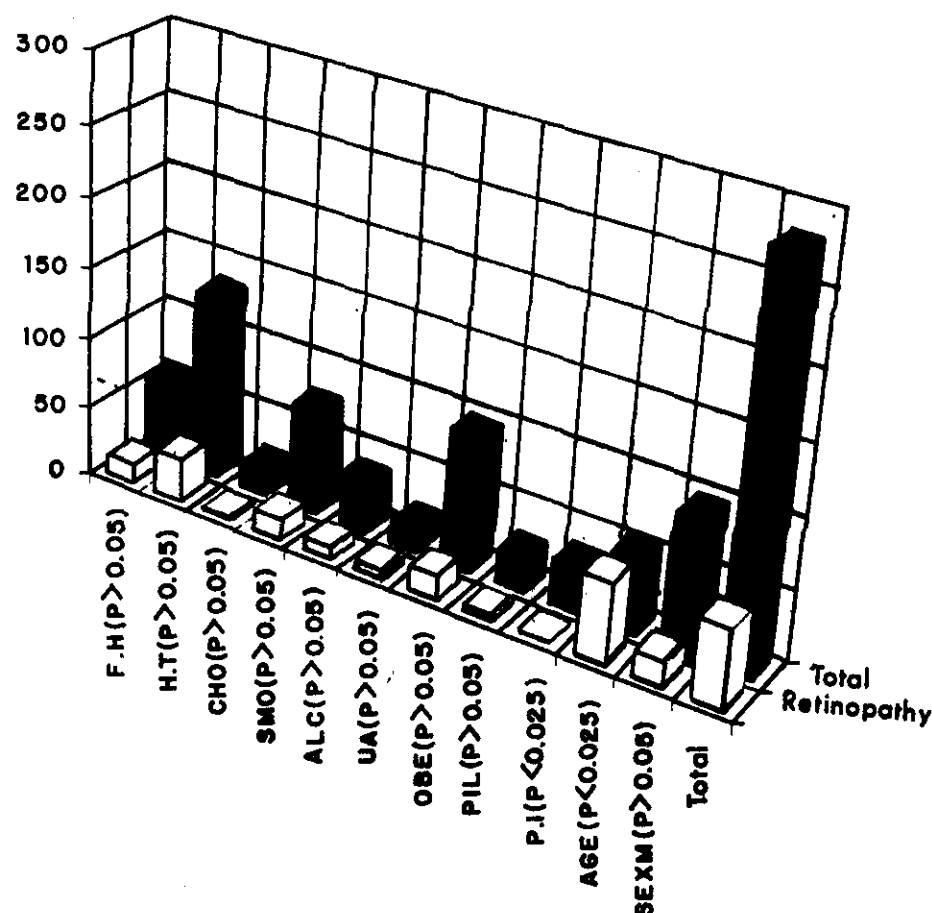


Fig. 1. Presence of risk factors and retinopathy

FH = family history; HT = hypertension; CHO = hypercholesterolaemia; SMO = smoking; ALC = alcohol; UA = hyperuricaemia; OBE = obesity; PIL = OC pill; PI = physical inactivity

correlated with duration of diabetes and age onset and higher serum creatinine levels [4]. Duration of diabetes was the most important risk, supported by the Wiscosin Epidemiologic study of Diabetic Retinopathy [5]. Risks for neuropathy in NIDDM in our study were hypertension, hypercholesterolaemia, smoking and age. Said reported that the most important risk for neuropathy was longer duration of diabetes [6].

Risks for nephropathy in NIDDM in our study were hypertension, alcohol, hyperuricaemia and age. Our understanding of nephropathy in NIDDM is complicated by uncertain duration of

NIDDM, and high prevalence of co-existing hypertension. Babolola reported that diabetes with hypertension had a greater degree of proteinuria [7].

Risks for CVA cerebrovascular accident (disease) were hypertension, physical inactivity, age and male sex. Kuller reported that there is a five fold risk of CVA in diabetics and hypertension is a major risk factor [8].

Risks for peripheral vascular disease in NIDDM were hypertension, physical inactivity and age. Alcolodo reported that hypertension and dyslipidaemia

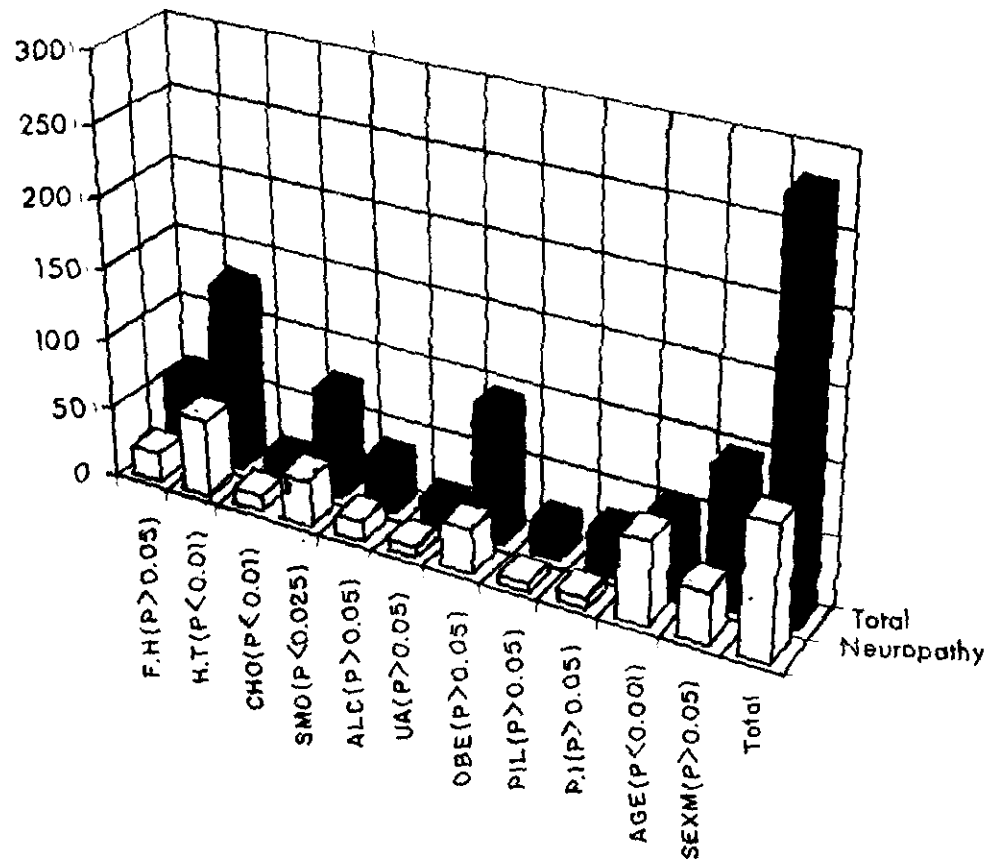


Fig. 2. Presence of risk factors and neuropathy

are important risks for peripheral vascular disease in NIDDM [9]. Migdolis reported that risks of peripheral vascular disease is related to the duration of disease [10]. Risks of ischaemic heart disease in NIDDM were OC pill, physical inactivity and age. Ford reported that coronary artery disease is leading cause of mortality among diabetes [11]. Ford reported that age, male sex, current smoking, hypertension and physical inactivity were associated with all cause mortality. Singer reported that the level of chronic glycaemia, determined by the measurements of glycosylated haemoglobin is an independent risk factor for coronary artery disease [12].

In IDDM significant risks for retinopathy in our study are age, duration

and fasting blood sugar. Similarly Krowleski reported that development of retinopathy in IDDM depends on duration of disease [13]. Klein reported that there is a relation between level of glucose control and prevalence and incidence of retinopathy [14].

Risks for nephropathy in IDDM were age, duration of disease and fasting blood sugar. In support of our findings Monske reported that there is association of both higher Haemoglobin A1C levels and number of years of diabetes in patients with nephropathy [15]. Beyer reported that in high risk group early identification of nephropathy included assessing glycaemic control, mean arterial pressure, urinary albumin excretion rate and glomerular hyperfiltration [16].

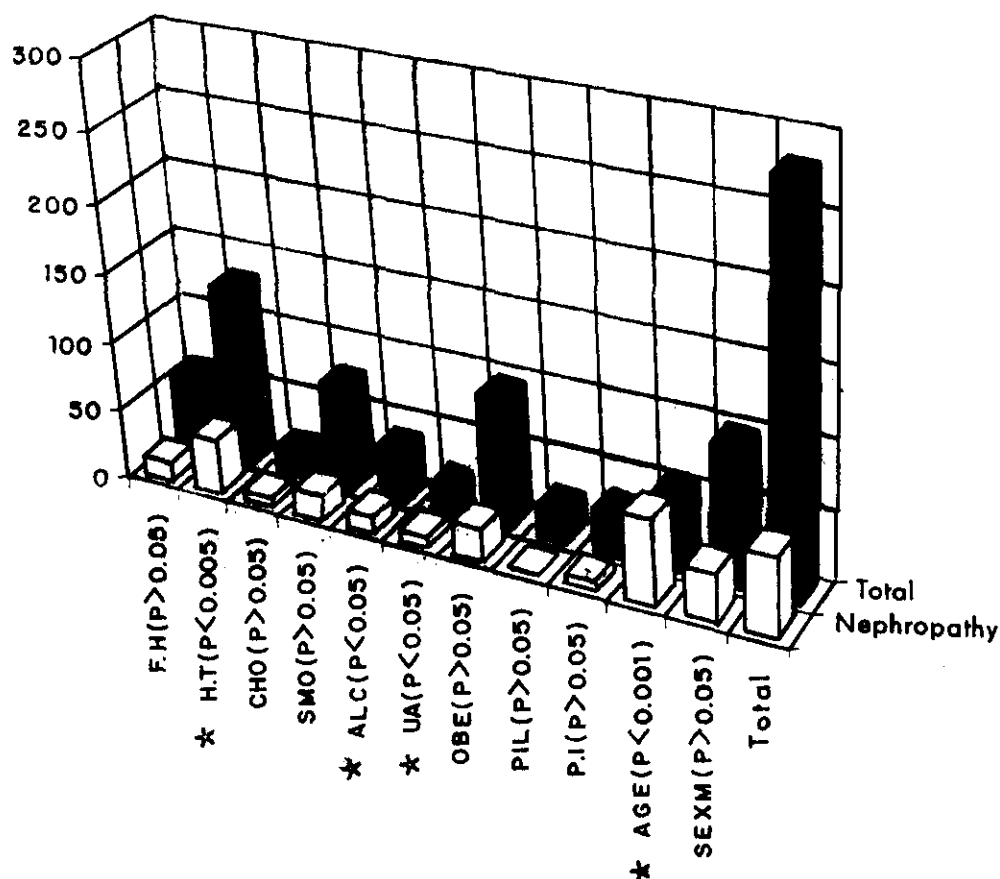


Fig. 5. Presence of risk factors and nephropathy

*Risk factors

CONCLUSION

Our study highlighted that age ie. (increasing age), indirectly reflecting duration of disease, is the main risk factor in the development of chronic complications in both NIDDM and IDDM. In NIDDM, hypertension enhances both micro and macrovascular complications and should be strictly controlled. In IDDM, the age, duration of disease and fasting blood sugar levels are significant risks in development of nephropathy and retinopathy. Thus effective glycaemic control is essential in their management.

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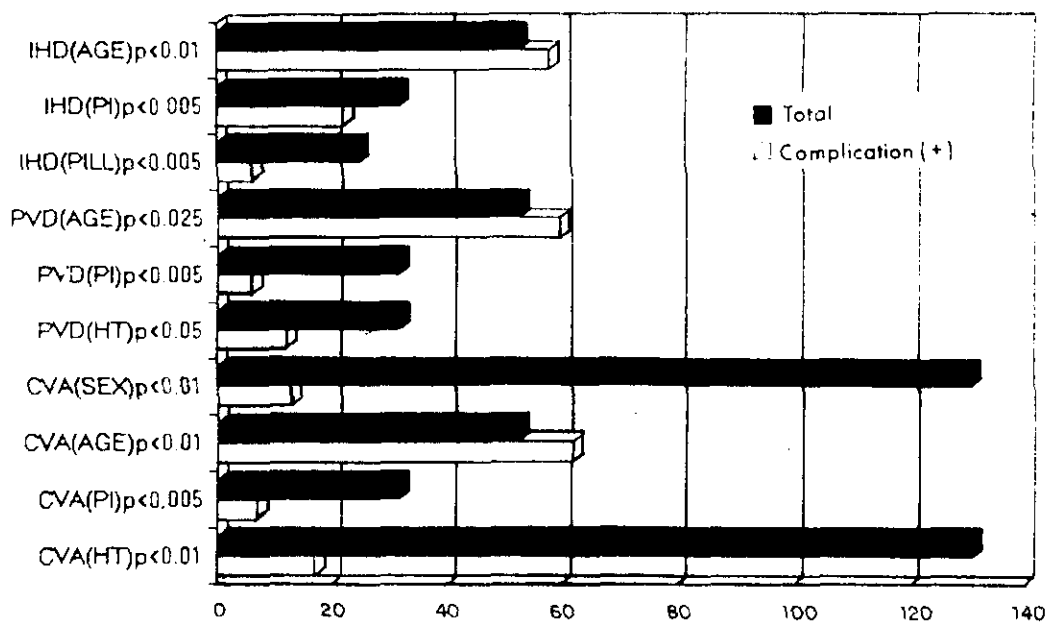


Fig. 4. Significant risk factors for atherosclerotic complications

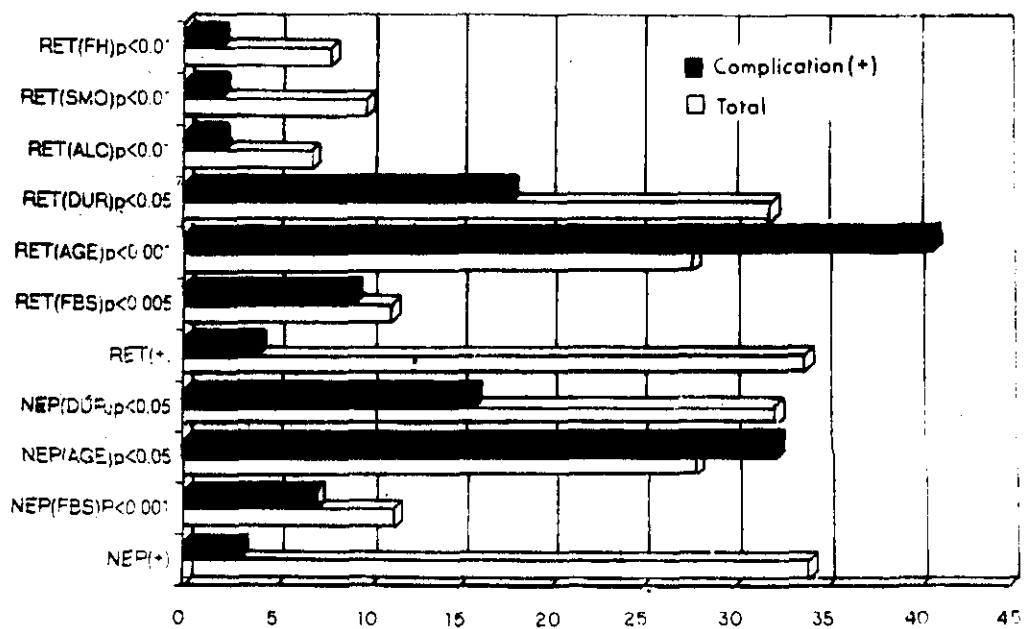


Fig. 5. Significant risk factors for complications in IDDM

RET = retinopathy; NEP = nephropathy; DUR = duration of diabetes; FBS = fasting blood sugar

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Anti-hepatotoxic substances from Eclipta alba (L.) Hassk. (Kyeik-hman)

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Three chemical compounds namely wedelolactone, demethyl wedelolactone and luteolin which had been reported to possess hepatoprotective activity were detected from Kyeik-hman (E. alba) grown in Myanmar. The active compounds were primarily identified by thin layer chromatography (TLC) by investigating R_f values and colour characteristics viewed under longwave ultra-violet (UV) light. The identification of above active substances was confirmed by UV spectral data analysis in comparison with standard marker or reference data reported in literature.

INTRODUCTION

An indigenous plant Kyeik-hman, botanically identified as Eclipta alba (L.) Hassk belonging to family Compositae or Asteraceae, is widely distributed throughout the whole Myanmar [1]. The leaves of the plant are claimed to be a good remedy for the treatment of jaundice by local people. Because of its traditionally reputed medicinal value for jaundice, Khin Ma Ma et al. [2] had attempted to evaluate and reported the hepatoprotective activity of liquid extracts of the leaves on carbon tetrachloride-induced acute liver damage using guinea pigs.

In 1986, Wagner et al. [3] had isolated and identified the responsible active principles from the leaves of the plant. The reported active substances were luteolin, wedelolactone and demethyl wedelolactone. Although the plant Kyeik-hman growing in Myanmar has been investigated pharmacognostically, detection of flavonoid and lactone compounds showing hepatoprotective property was not done yet [4]. The present study was attempted to detect and identify the reported active principles from the leaves of E. alba collected from

Yangon area, by extracting with locally available solvent ethanol.

MATERIALS AND METHOD

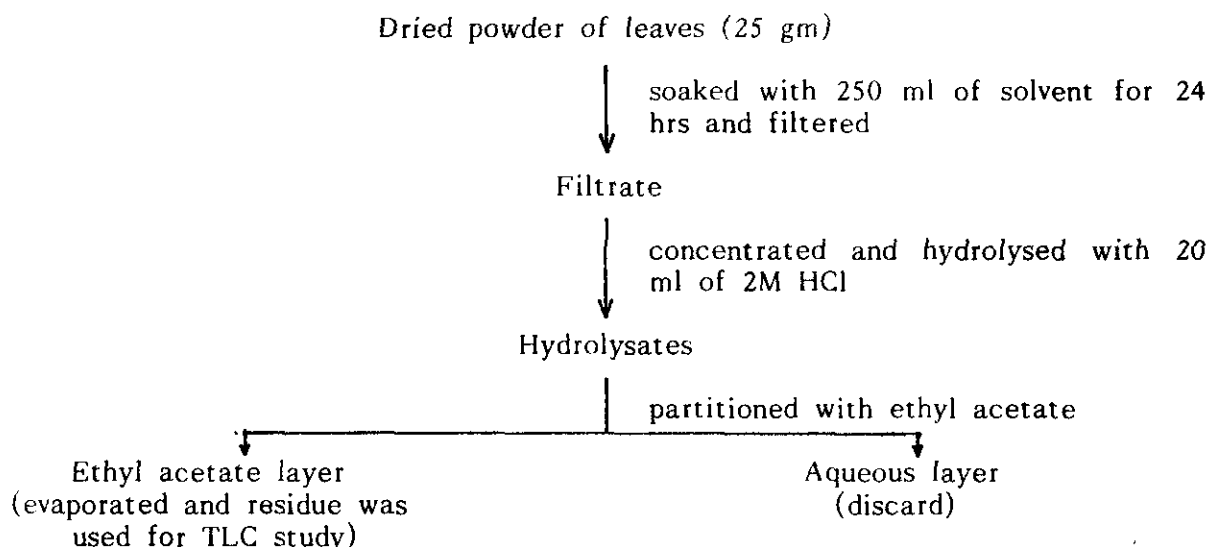
The plant materials were collected from various localities such as Hlaing, Kyimyindaing and South Okkalapa. The air-dried leaves were pulverized using grinding machine. Solvents used for the extraction were methanol (BDH), 95% ethanol (local) and 70% ethanol. Precoated cellulose plates (Schleicher & Schvell Co.) and precoated silica gel plates (Sigma Chemical Co.) were used for TLC studies. Absorption spectra of analysed compounds were measured by means of UV-VIS spectrophotometer (model Hitachi 100-50).

The extraction procedures for the flavonoid compound, luteolin [5] and lactone compounds, wedelolactone and demethyl wedelolactone [3] are described by the flow charts 1 and 2 respectively. Three solvents used in the extraction were methanol, 95% ethanol and 70% ethanol.

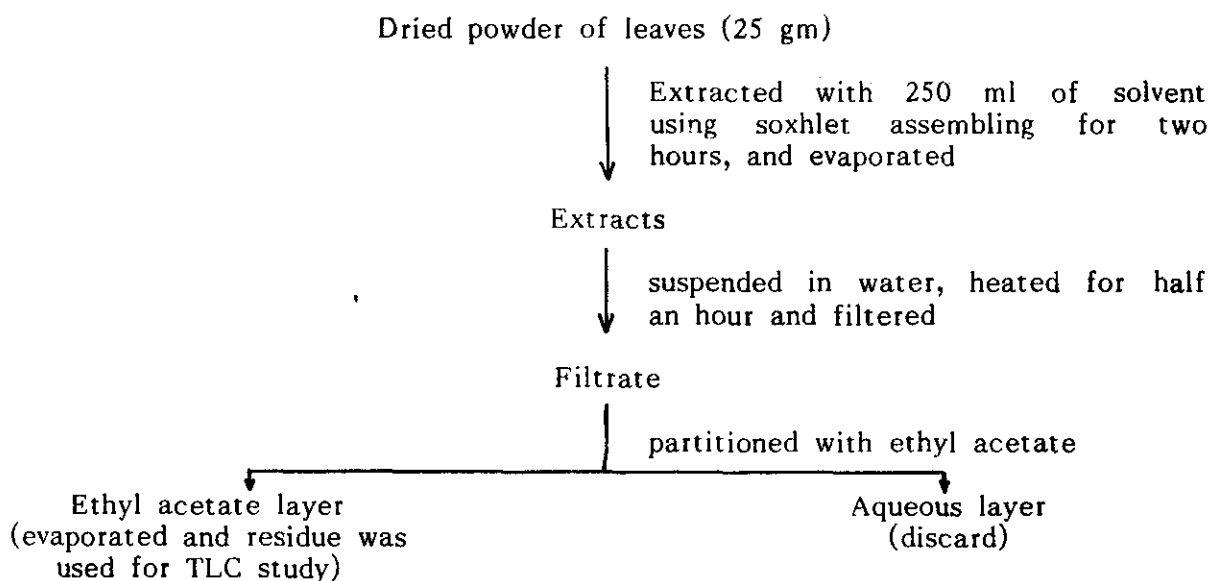
RESULTS AND DISCUSSION

TLC study of extract obtained through

Flow chart 1. Extraction of flavonoid



Flow chart 2. Extraction of lactones



flow chart 1 indicated a similar spot with the standard luteolin. In another extract obtained through flow chart 2, two spots which correspond to the reported data of wedelolactone and demethyl wedelolactone were detected respectively. The chemical substances similar to hepatoprotective compounds were collected from the chromatogram and eluted as single constituent. UV

spectral data were measured and compared. The identification of hepatoprotective substances in the *E. alba* extracts was confirmed according to the data shown in the Tables. Schematic diagrams of chromatograms and spectrum of analysed compounds are described in figures 1,2,3,4 and 5. It was found that analysed substances were detected in all the

Table 1. Experimental data for identification of luteolin

Sample	Rf value			Colour under UV		Absorption spectrum
	Forestal	50% HOAc	BAW	Without treatment	Treated with NH_3	
Std. luteolin	0.71	0.52	0.80	Gray	Green Yellow	255,268,350 (nm)
Analysed compd. from <i>E. alba</i>	0.71	0.52	0.80	Gray	Green Yellow	255,268,350 (nm)

Forestal = Acetic acid - Con. hydrochloric acid - Water (30:3:10)

50% HOAc = 50% Acetic acid

BAW = n-Butanol - Acetic acid - Water (4:1:5), top layer

Table 2. Experimental data for identification of lactones

Sample	Rf values		Colour under UV	Absorption spectrum
	TAF	$\text{CH}_2\text{Cl}_2\text{-MeOH-H}_2\text{O}$		
Wedelolactone reported data	0.63	0.58	Bright blue	209,249,302,351
Analysed compd. from <i>E. alba</i>	0.63	0.58	Bright blue	209,249,302,351
Demethyl wedelolactone reported data	0.50	0.48	White blue	209,249,302,351
Analysed compd. from <i>E. alba</i>	0.50	0.47	White blue	210,249,302,351

TAF = Toluene - Acetone - Formic acid (11:6:1)

$\text{CH}_2\text{Cl}_2\text{-MeOH-H}_2\text{O}$ = Ethylene dichloride - Methanol - Water (14:9:4)

extracts indicating that hepatoprotective compounds could be extracted with locally available alcohol from the plant Kyeik-hman.

Kyeik-hman is one of the famous Myanmar indigenous medicinal plants, reputed for various diseases. Now this plant had been scientifically investigated and proven for its medicinal values regarding the hepatoprotective activity. In Myanmar, the plant Kyeik-hman was experimentally investigated pharmacognostically by Yi Yi Than in 1973, pharmacologically using carbon tetrachloride-induced acute liver damage guinea pigs by Khin Ma Ma *et al.* in 1978, and chemically by me now. Such a scientifically proven medicinal plant

would thus be useful for traditional practitioners and most of the local people who believe in natural medicinal plants, and persons who are interested in its medicinal value to carry out further scientific research works.

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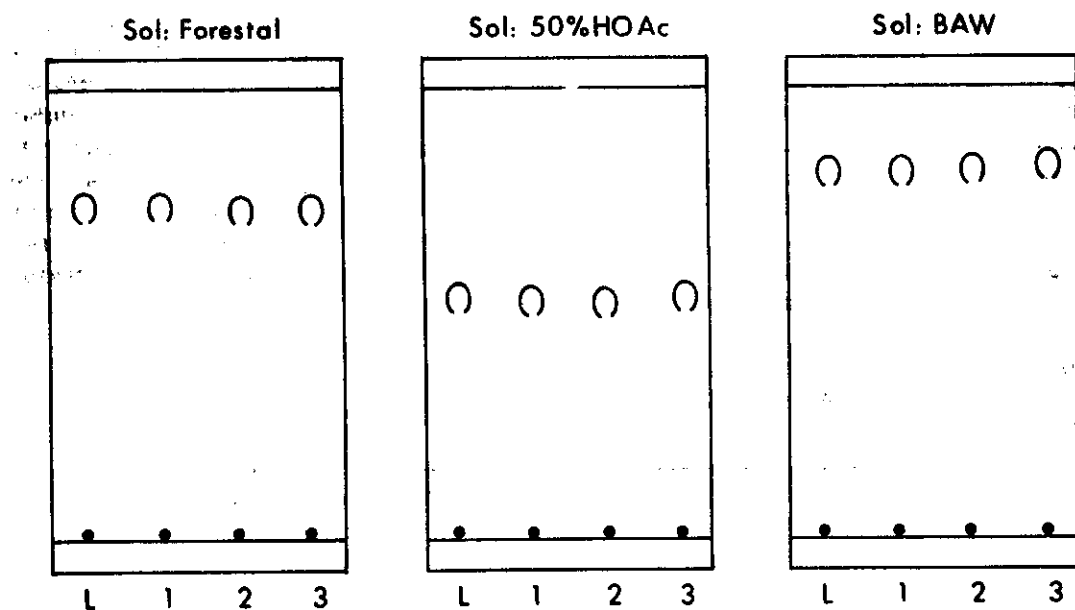


Fig. 1. Chromatogram of flavonoids

- L = Standard luteolin
- 1 = Methanolic extracts of E. alba
- 2 = 95% ethanolic extracts of E. alba
- 3 = 70% ethanolic extracts of E. alba

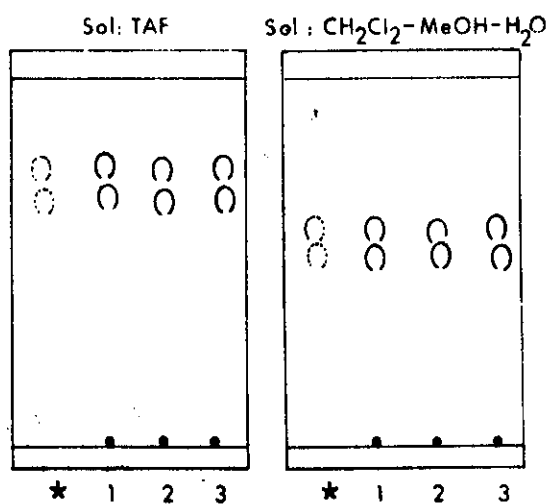


Fig. 2. Chromatograms of lactones

- * = Reported data of wedelolactone and dimethyl wedelolactone
- 1 = Methanolic extracts of E. alba
- 2 = 95% ethanolic extracts of E. alba
- 3 = 70% ethanolic extracts of E. alba

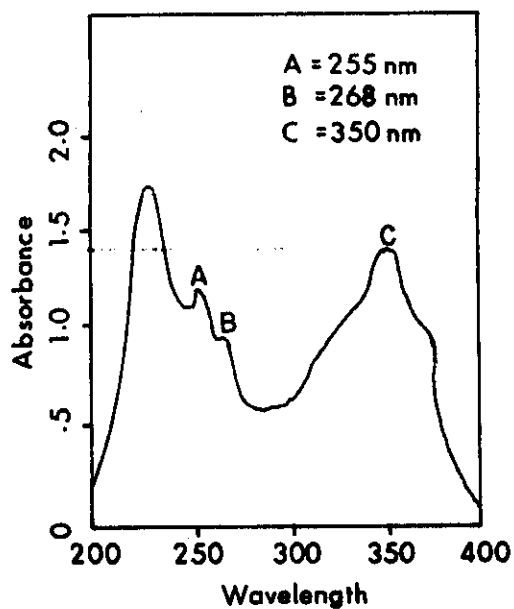


Fig. 3. Spectrum of isolated luteolin

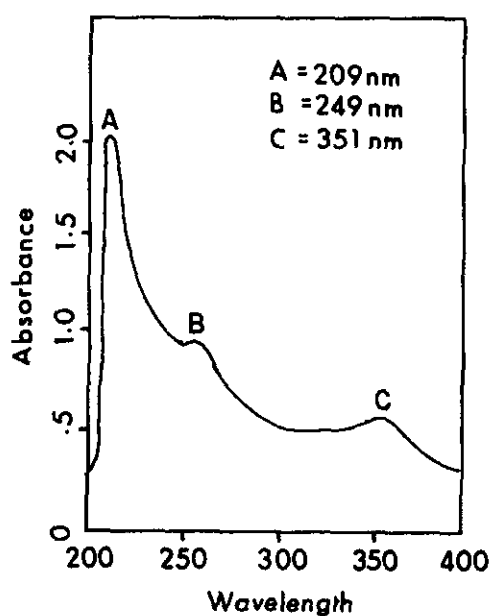


Fig. 4. Spectrum of isolated wedelolactone

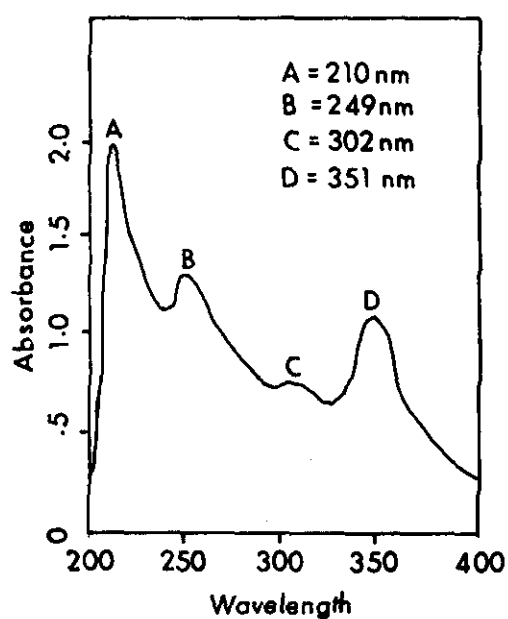


Fig. 5. Spectrum of isolated demethyl wedelolactone

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The peripheral lymphocyte mitogenic responses of apparently healthy Myanmar blood donors

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The responses of peripheral lymphocytes to mitogens namely, phytohemagglutinin (PHA) and lipopolysaccharide (LPS) were determined in 50 apparently healthy blood donors. The stimulation indexes of PHA and LPS of male and female donors were calculated. Though there was a significant difference ($P < 0.001$) between the sexes to PHA response, no significant difference was found in LPS response. Also the mitogen responses had no correlation with the age and the number of T and B lymphocytes in the peripheral blood.

INTRODUCTION

In normal person, cells of the immune system will protect the individual by a series of changes in both the T and B lymphocytes and among them blastogenesis of these lymphocytes to specific antigens and mitogens is one of the earliest and detectable responses. For T cell function T cell mitogens like PHA was used and similarly for B cell function, B cell mitogens such as LPS was used. At present these mitogenic responses were regarded as the functional status of the lymphocytes and reflect the immunological competency or deficiency of the individual. This study was carried out to determine the mitogenic responses of the peripheral lymphocytes of the apparently healthy Myanmar blood donors.

MATERIALS AND METHODS

Study population

Fifty apparently healthy Myanmar blood donors of both sexes, age ranging from 15 to 55 years, were studied. Five milli litre of heparinized blood (10 IU heparin/ml of blood) was collected from each donor.

Mitogens

PHA and LPS (Sigma Co.) were used as T and B lymphocytes mitogens respectively. The optimum concentrations of PHA and LPS for maximum mitogenic responses were tested in 3 days culture with various concentrations. A graph (Fig. 1) was plotted and optimum concentration calculated. It was found to be 12.5 mg/ml for PHA and 6.25 mg/ml for LPS.

Lymphocyte isolation and determination of blastogenesis

5 ml of heparinized blood was diluted with phosphate buffered saline (PBS) pH 7.4 and layered over 4 ml of Ficoll-hypaque (Pharmacia Fine Chemicals) and centrifuged at 1500 rpm for 30 minutes at room temperature. After centrifugation, mononuclear cells-rich layer was drawn and washed three times in cold PBS at 1500 rpm for 5 minutes at 4°C. Washed mononuclear cells were resuspended in 1 ml of RPMI 1640 medium (Gibco Co.). 20 ml of cell suspension was taken and mixed with equal volume of 2% Trypin blue and counted in hemocytometer for the determination of total mononuclear cells as well as viable cells. More than 95% viable

cells were obtained in each preparation. Appropriate dilution of the mononuclear cells was made to obtain 2.5×10^6 cells/ml in RPMI 1640 supplemented with 5% fetal bovine serum (Irvine Scientific), penicillin and streptomycin. 200 μ l of 2.5×10^6 cells/ml were delivered into 96-wells microcultured plate, in quadruplicate for each sample. 20 μ l of the optimum concentration of either PHA or LPS was added and incubated at 37°C with 5% CO₂ for 3 days. Then (³H) thymidine 1mCi/well was added and pulsed for 3 hours. The cultured cells were harvested by cell harvester on to fibre glass filter discs and allowed to dry. Amount of ³H thymidine incorporated was counted in the liquid scintillation counter (Nuclear Enterprise LSM 1) after dissolving the fiber glass filter discs in scintillation cocktail. For control, cells were cultured with PBS. The stimulation index (SI) was calculated as follows:

$$SI = \frac{\text{Mean of test count per minute}}{\text{Mean of control count per minute}}$$

Total leukocyte count and differential leukocyte count were determined by the manual counting. The T lymphocyte count was estimated by the sheep erythrocyte resetting technique. The statistical significance was determined by Student's 't' test.

RESULTS

Fig. 1 shows the optimum concentration curves of the PHA and LPS at various concentrations under the standardized cultural conditions.

Table 1 reveals the mitogenic response of PHA and LPS for male and female donors. There was a significant difference ($P < 0.001$) between male and female in PHA mitogenic response. But there was no significant difference between the two sexes in LPS mitogenic response. Also there was no

significant difference between young and old subjects in both PHA and LPS mitogenic responses. Table 2 shows the total leukocyte count, total lymphocyte count and T lymphocyte count of male and female donors. There was no significant difference in these data between the two sexes.

DISCUSSION

The *in vitro* response of the peripheral lymphocytes to mitogens is widely used to evaluate cell-mediated immunity especially in distinguishing the response of patients with immune deficiency disease from normals [1,2,3]. Many factors such as cell concentration, types of cells, serum supplement, pH, atmospheric conditions, batch and concentration of mitogens and duration of culture influence the mitogenic response [4,5]. We had standardized the assay conditions that would provide the optimum mitogenic response with the aims that at maximum stimulation immune suppression could be differentiated from normal immune competent control.

Since there was a significant difference ($P < 0.001$) between male and female to the PHA response, the number of T lymphocyte in the peripheral blood was also determined. As depicted in Table 2 there was no correlation between the absolute number or percentage of T and B lymphocytes subpopulation with the mitogenic responses. Pisciotto *et al.* [6] had reported that there was no significant difference between sex and race to PHA response but they had not mentioned the details of their cultural conditions and had used morphological method to detect blastic transformation.

We had also evaluated the mitogenic responses in relation to the age of the donor, but there was no significant

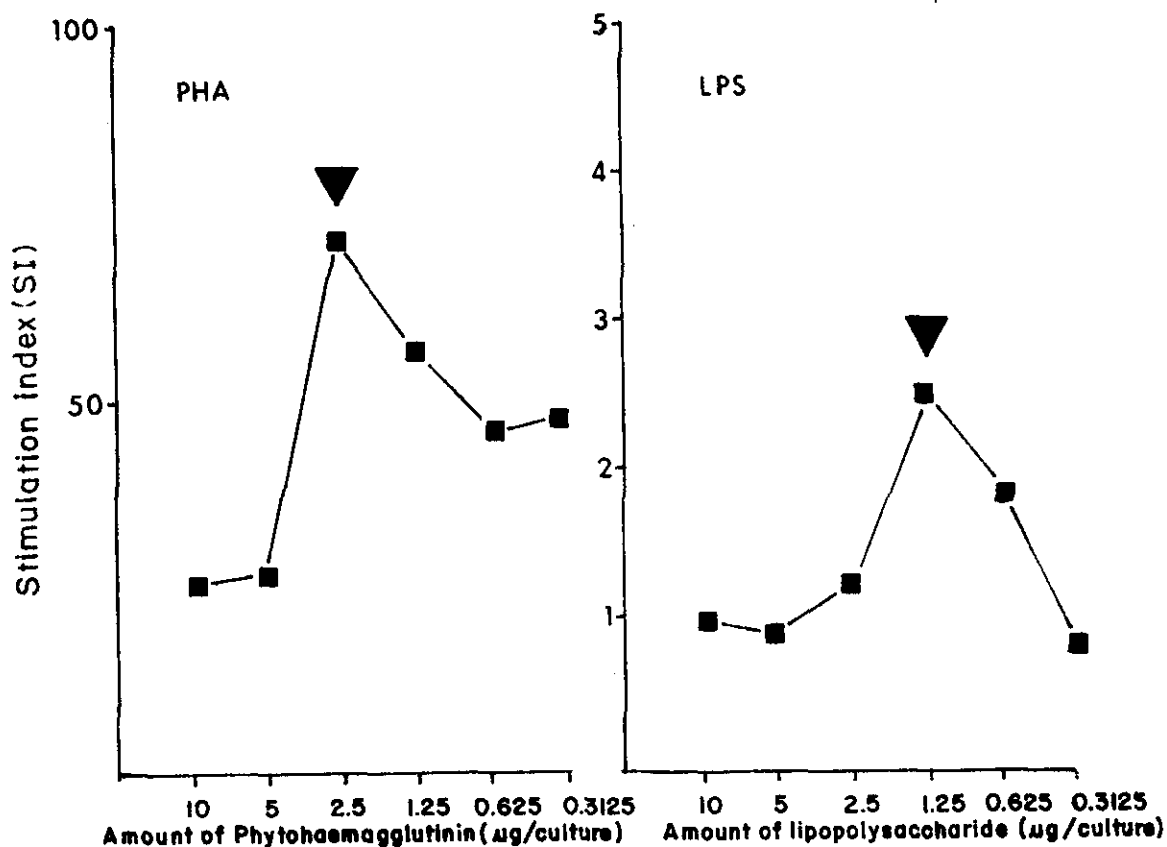


Fig. 1. Optimal concentration of mitogens

Table 1. The stimulation indexes of mitogenic responses of 50 male and female blood donors, Yangon 1992

Age group	Mitogens	Stimulation index of male donors		Stimulation index of female donors	
		No.	Mean $\pm$ 1SD	No.	Mean $\pm$ 1SD
15-35	PHA	13	30.14 $\pm$ 13.17	14	12.41 $\pm$ 5.61
	LPS		1.77 $\pm$ 0.69		1.20 $\pm$ 0.34
36-55	PHA	12	38.23 $\pm$ 17.23	11	15.28 $\pm$ 8.27
	LPS		1.37 $\pm$ 0.40		1.50 $\pm$ 0.74
15-55	PHA	25	34.02 $\pm$ 15.49	25	13.68 $\pm$ 6.90
	LPS		1.56 $\pm$ 0.60		1.33 $\pm$ 0.56

PHA = Phytohemagglutinin

LPS = Lipopolysaccharide

difference between those below and above 36 years of age. Since we had very few donors above 50 years old that we could not determine

those below and above 50 years old individuals. Westring *et al.* [7] had mentioned that the mitogenic response decreased after 50 years of age but

Table 2. Total leukocyte, total lymphocyte and total T cells ($\times 10^9/L$) of 50 male and female blood donors

	Male donors		Female donors	
	No.	Mean $\pm$ 1SD	No.	Mean $\pm$ 1SD
Total WBC	25	6.60 $\pm$ 1.70	25	9.11 $\pm$ 1.70
Total lymphocyte	25	2.70 $\pm$ 0.73	25	3.40 $\pm$ 1.02
Total T cells	25	1.16 $\pm$ 0.35	25	1.47 $\pm$ 0.83

they had used morphological method for the detection of blastogenesis. Bernhard *et al.* [8] had studied mitogenic responses of peripheral blood lymphocytes and they were also unable to distinguish differences in response between young and old individuals.

ACKNOWLEDGEMENT

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Direct relaxant effect of some plant extracts on isolated tissue models

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The effects of two different extracts of three medicinal plants namely *Piper betle* L. (Kun), *Coleus aromaticus* Benth. (Ziyar-ywet-htu) and *Ageratum conyzoides* L. (Khway-thay-pan) leaves on the smooth muscles of guinea pig and rat were tested *in vitro* using isolated segments of trachea, intestine and uterus. The extracts inhibited the contractions of guinea pig tracheal muscle induced by carbachol and histamine stimulation. The alcoholic extracts also inhibited the spontaneous movement of guinea pig ileum induced by histamine stimulation. Furthermore, the alcoholic extracts inhibited the contractions of rat uterus induced by 5-HT stimulation. These data suggest that the alcoholic extracts have antihistaminic and anti 5-hydroxytryptamine effects and the active principle may probably resides in the saponin glycoside component.

INTRODUCTION

The leaves of the plants *Piper betle* Linn. (Kun), *Coleus aromaticus* Benth. (Ziyar-ywet-htu) and *Ageratum conyzoides* Linn. (Khway-thay-pan), have been widely employed to treat conditions such as catarh and bronchitis, inflammation, boils, ear pain, cough and as an expectorant [1].

A survey of literature indicates that no systematic investigation on the pharmacology of the plant extracts have been carried out. Considering its widespread and successful use in bronchial asthma in the Ayurvedic system of medicine, it was thought useful to elucidate its action and possible mechanism of action.

MATERIALS AND METHODS

Preparation of plant extracts

A total number of three reputed anti-asthmatic and expectorative Myanmar medicinal plants used in

this study were prepared according to the classical text [2]. Voucher specimens have been deposited at the Pharmacognosy Section, Pharmacology Research Division, Department of Medical Research, Yangon, Myanmar. Qualitative identification tests of the chemical constituents present in the effective tested samples were conducted according to the standard procedures as stated in Trease and Evans [3] and Pharmacopoeia [4].

Test of plant extracts on isolated tracheal chain preparation

The isolated tracheal chain preparation of guinea pig was employed as described by Castillo and De Beer [5] and Agel and Hadidi [6]. The trachea was removed from the guinea pig immediately after sacrifice and was kept moist in aerated Krebs Hanseleit's solution. Segments of trachea, each containing of 3-4 rings of cartilages were dissected. Four to five such segments of tracheal rings were tied with a cotton thread in such a

way that the muscular portions were alternately orientated on opposite sides. The strip was mounted in an organ bath of 50 ml capacity which was maintained at 37°C and aerated with 95% oxygen and 5% carbondioxide. One end of the strip was attached to the gas supplying tube and the other to a transducer (Myograph transducer type A, E & M Instrument, U.S.A.) and responses were recorded on a physiograph (Narcotrace-40, Narco Bio-Systems, U.S.A.). An appropriate passive tension was applied, and the strip was allowed to equilibrate for half an hour. The response of the tracheal muscle to each dose of the test extract was recorded for 5 mins. The test substance was then washed out and the tissue was allowed to rest for 15 mins or more until the tissue recovered to the base-line. The same procedure was carried out on the strip which was allowed to contract by adding carbachol (0.25 mg/ml bath conc.) to the organ bath. Thus, the effect of the test extract had been tested on normal tissue as well as the stimulated tracheal muscle.

Test of plant extracts on the isolated ileal preparation

A piece of terminal ileum obtained from a guinea pig of either sex was mounted in 50 ml bath containing oxygenated Tyrode's solution at 37°C using the method of Kitchen [7]. The tracing of the ileal contraction was recorded on the physiograph as mentioned in the former test. The effect of histamine (2 ug/ml bath conc.) only and alcoholic extract (2 mg/ml bath conc.) together with histamine were observed on the smooth muscles of the isolated ileum preparation.

Test of plant extracts on isolated uterus preparation

One horn of the rat uterus was

dissected out and mounted in the isolated bath containing aerated de Jalon's solution at 32°C as described by Kitchen [7]. The tracing of the uterus contraction was recorded on the physiograph as mentioned before. The effect of 5-hydroxytryptamine (2 ug/ml bath conc.) only and alcoholic extract (2 mg/ml bath conc.) together with 5-HT were observed on the smooth muscles of the isolated uterus preparation.

RESULTS

Qualitative tests of the watery extracts

The results from the qualitative identification tests on the effective aqueous and alcoholic extracts of three plants are shown in Table 1.

Effect on trachealis muscle

Tracing sample of relaxing response of three plant extracts on guinea pig tracheal preparations are shown in Fig. 1. It was found that the three aqueous extracts produced relaxation effect on normally contracted trachealis muscle.

When the aqueous extracts were added to the organ bath after stimulating the trachealis muscle with carbachol, the three extracts also inhibited the contraction. The magnitude or height of contraction in mm in its original scale as recorded by the physiograph is shown in Fig. 2.

The effect of the effective plant extracts on the isolated tracheal chain preparation is shown in Table 2. The plants tested inhibited the contraction induced by the contractile agent ($p < 0.025$). When the aqueous extracts were added to the organ bath after stimulating the trachealis muscle with histamine (0.4 mg/ml bath conc.), the three extracts also inhibited the contraction. The magnitude or height of contraction in mm

Table 1. Qualitative tests of different types of extracts of Piper betle L. (Kun-ywet), Coleus aromaticus B. (Ziyar-ywet-htu) and Ageratum conyzoides L. (Khway-thay-pan)

Samples	Alkaloid	Amino acid	Flavonoid	Glycoside	Saponin	Steroid	Terpenoid
Kun							
Aqueous extract	-	+	-	+	+	-	-
70% alcoholic extract	+	+	-	+	+	+	-
Khway-thay-pan							
Aqueous extract	+	+	-	+	+	-	-
70% alcoholic extract	+	+	-	+	+	-	-
Ziyar-ywet-htu							
Aqueous extract	-	+	-	+	+	-	-
70% alcoholic extract	-	+	-	+	+	-	-

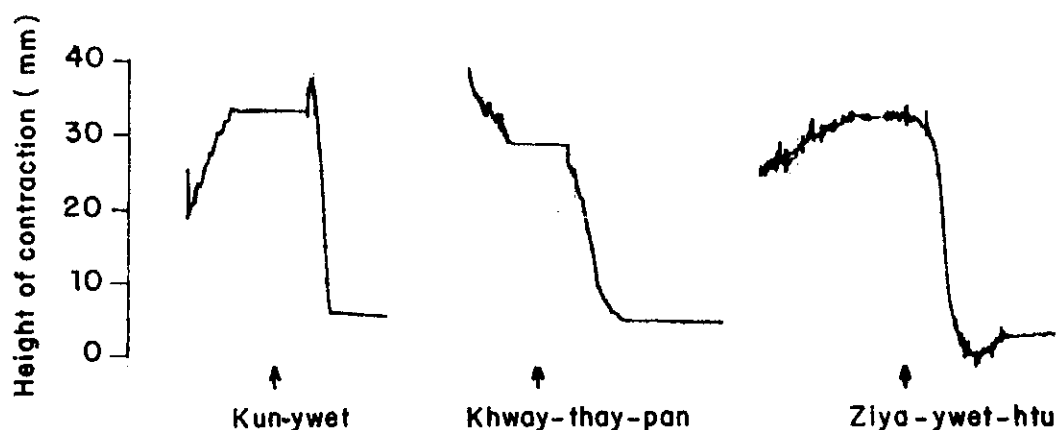


Fig. 1. Tracing samples of effect of aqueous extracts on normally contracted tracheal chain preparations

The magnitude of the illustration is in its original scale as recorded by the physiograph as done on guinea-pig tracheal chain preparation. Arrows show the points of aqueous extracts (2 mg/ml bath conc.) administration.

is in its original scale as recorded by the physiograph (Fig. 3).

Effect on isolated ileum

Tracing sample of isolated ileum contraction after addition of histamine alone and plant extracts together with histamine is shown in Fig. 4. The alcoholic extracts of Kun, Ziyar-ywet-htu and Khway-thay-pan were found to block the excitatory effects of histamine on isolated ileal muscle. The three alcoholic extracts were found to block the histamine (2

ug/ml) response completely. The excitatory effect of histamine could be restored after washing.

Effect on isolated uterus

The three alcoholic extracts of the effective plants had antispasmodic effect on rat uterus similar to that on guinea pig ileum (Fig. 5). All the three extracts blocked the response of 5-HT (2 ug/ml bath conc.). The blockade of 5-HT response by the above three extracts were found to be reversible.

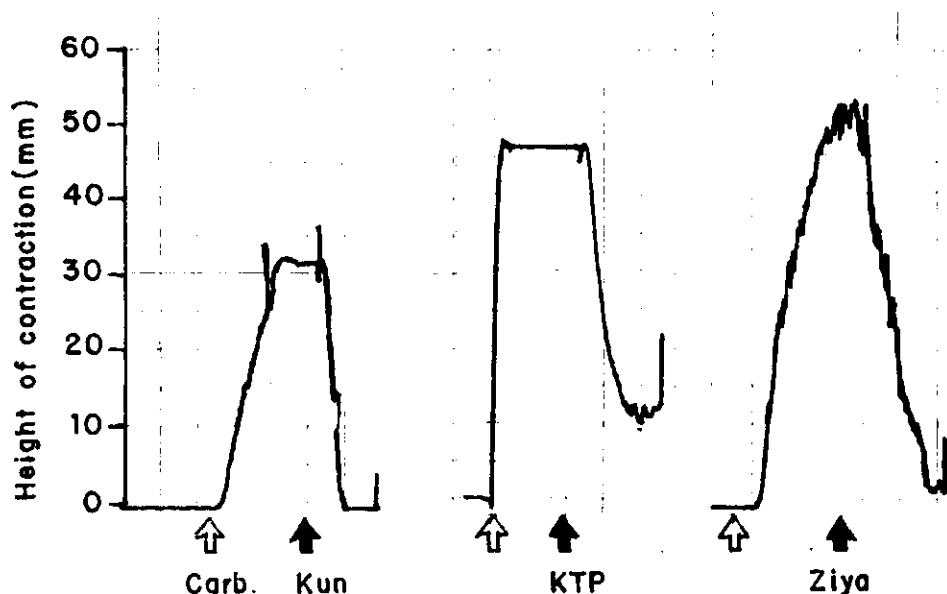


Fig. 2. Tracing sample of effect of aqueous extracts on carbachol-induced contracted tracheal chain preparations

The magnitude of the illustration is in its original scale as recorded by the physiograph as done on guinea-pig tracheal chain preparation. Arrows show the points of carbachol (0.25 mg/ml bath conc.) and aqueous extracts (2 mg/ml bath conc.) administrations.

Table 2. Relaxation effect of aqueous extracts of three plants on isolated carbachol-induced guinea pig tracheal chain preparations

Height of contraction after addition of carbachol, 0.25 mg/ml bath conc. (mm)	Height of contraction after addition of aqueous extracts, 2.0 mg/ml bath conc. (mm)	Relaxation response (%)	Difference p
Kun (n = 10) 48.0 ± 6.3	9.5 ± 6.3	80.2	p < 0.005
Khway-thay-pan (n = 6) 45.0 ± 8.1	8.0 ± 3.3	82.2	p < 0.005
Ziyar-ywet-htu (n = 6) 52.0 ± 5.7	23.0 ± 9.9	55.8	p < 0.005

n = denotes number of observations tested

DISCUSSION

The aetiology of bronchial asthma is still obscure, however, histamine and other pharmacologically active endogenous substances like slow reacting substance of anaphylaxis (SRS-A),

platelet activation factor (PAF), eosion eosinophil chemotactic factor of anaphylaxis (ECF-A), prostaglandins (PGS) and bradykinin are considered to cause obstruction of the air passage and other local reactions as stated by Mcfadden and Austen

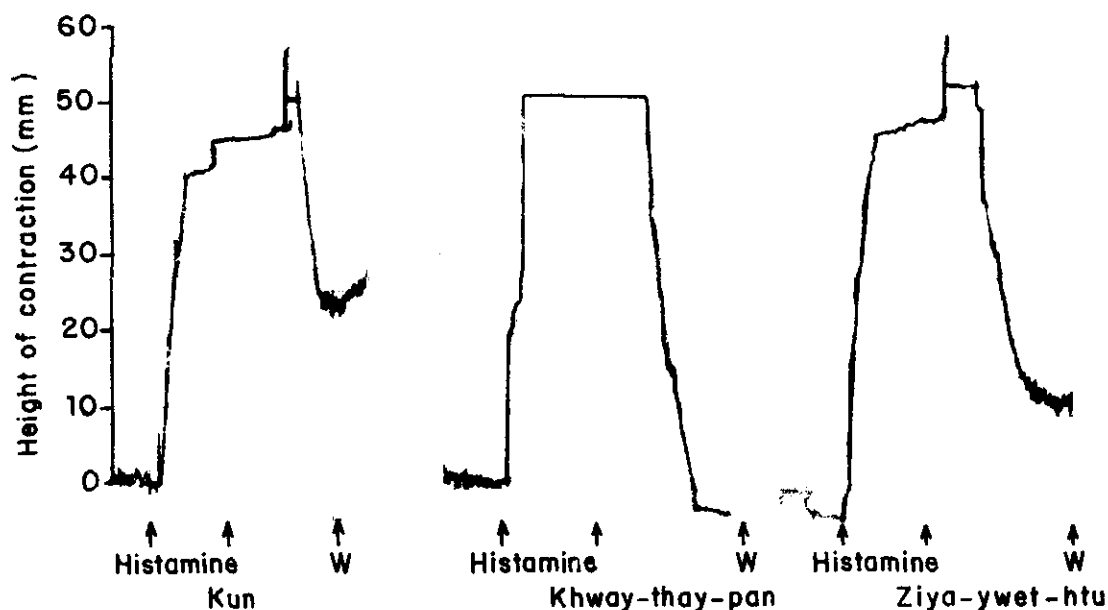


Fig. 3. Tracing samples showing the effect of three plant extracts on the contractile responses of isolated tracheal chain of guinea pig to histamine. The magnitude of the illustration is in its original scale as recorded by the physiograph. Arrows show the point of histamine (20 ug/ml bath conc.) and plant extract (2 mg/ml bath conc.) administration into the muscle bath.

[8]. 5-HT also causes broncho-constriction in many experimental animals, but this response is uncommon in men except in asthmatic patients as reported by Douglas [9]. It was observed that the three aqueous extracts produced relaxation effect on normal tracheal tissues. These extracts also relaxed the carbachol-induced and histamine-induced trachealis muscles respectively. The results of the present study have demonstrated that all the three alcoholic extracts possess potent anti-5-HT and anti-histaminic activities as revealed by blockade of histamine-induced contractions of guinea pig ileum; 5-HT induced contractions of rat uterus. The yield of watery extracts from fresh leaves of Kun, Khway-thay-pan and Ziyar-ywet-htu were 4.0 g, 1.5 g and 2.1 g per 100 g fresh leaves whereas the yield of alcoholic extracts from the stated three plants were 4.5 g, 2.1 g and

2.2 g per 100 g fresh leaves respectively. It was reported that Kun-ywet was used in respiratory catarrh. It dispels flatus, stop coughing, reduces inflammation, alleviates itching [1]. The leaves of Ziyar-ywet-htu were used in cases of aphthous stomatitis. Eugenol and methyleugenol were also found to occur in its oil [10]. Khway-thay-pan extracts showed anti-juvenile hormone-like activity against laboratory colonized larvae and adult female mosquitoes [11]. All the three watery extracts and alcoholic extracts were tested for chemical constituents present in the samples. It was observed that mainly alkaloids, glycosides and saponins were present. So the possible active principles are saponin glycosides which could be extracted with 70% alcohol. Thus the beneficial effect of these plants in bronchial asthma and as cough expectorant appears to be due to its antihistaminic and anti-5-HT properties.

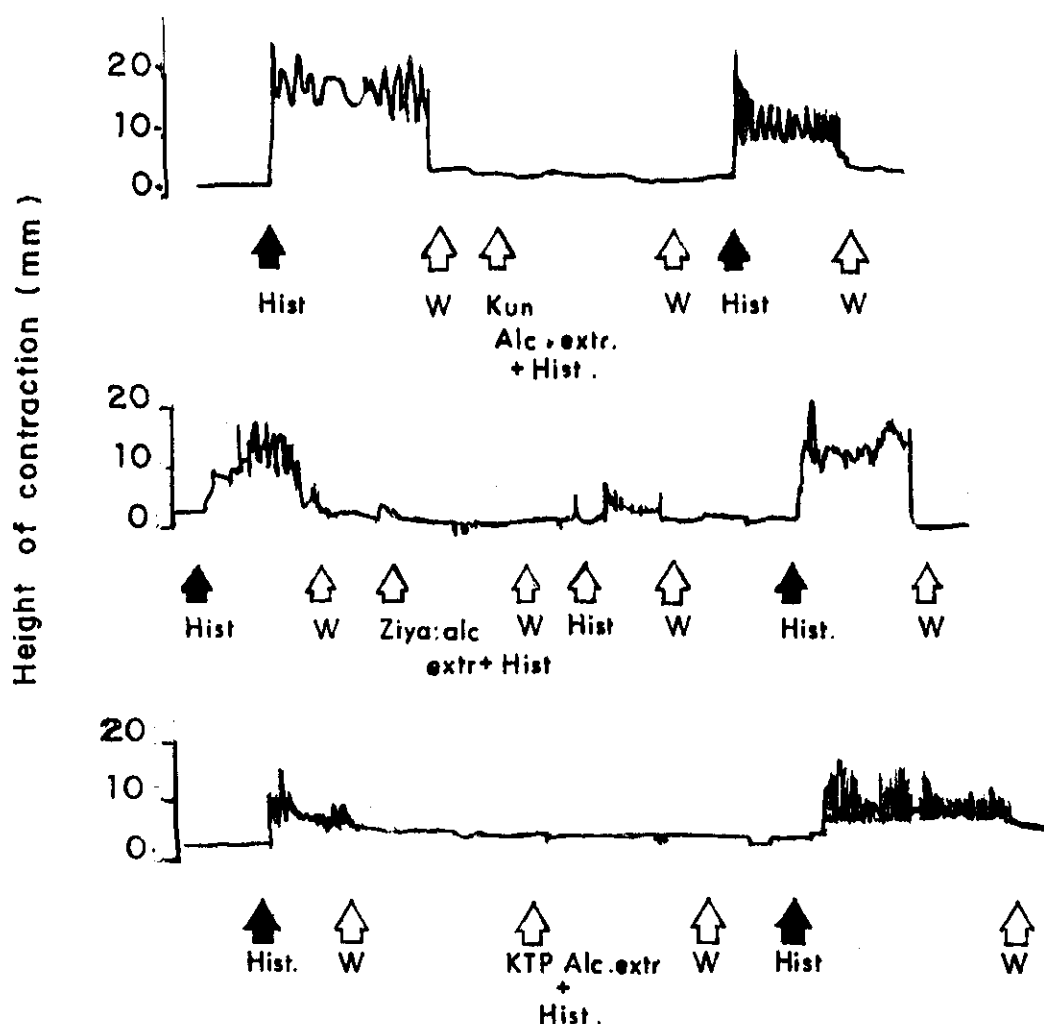


Fig. 4. Tracing samples of effect of alcoholic extracts on spasmogenic effect of histamine on isolated guinea-pig ileal preparations.

The magnitude of the illustration is in its original scale as recorded by the physiograph as done on guinea-pig ileal preparations. Arrows show the points of histamine (2 ug/ml bath conc.) and alcohol extracts (2 mg/ml bath conc.) administrations.

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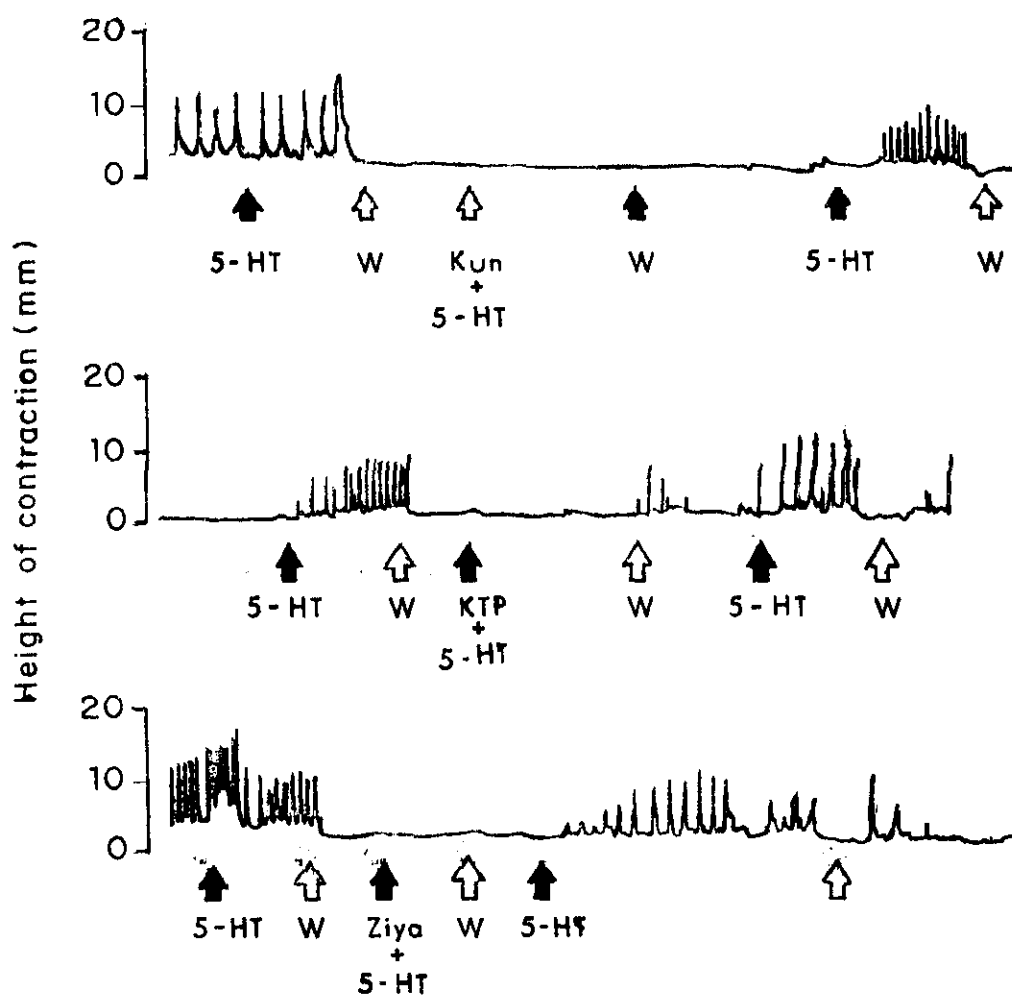


Fig. 5. Tracing samples of effect of alcoholic extracts on spasmogenic effect of 5-HT on isolated rat uterus preparations

The magnitude of the illustration is in its original scale as recorded by the physiograph as done on rat uterus preparation. Arrows show the points of 5-HT (2 ug/ml bath conc.) and alcohol extracts (2 mg/ml bath conc.) administrations.

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Growth of thalassaemic children in Myanmar

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The growth of thalassaemic children was studied at the Day-Care-Room in Yangon Children's Hospital. Most of these patients came to the Day-Care-Room for transfusion only, when their pretransfusion haemoglobin level became very low (range 3-8.2g/dl, median 5.0g/dl). Their spleens and livers were moderate to markedly enlarged. The percentage of children who had below median -2SD for weight and height for age were 68.8% and 72.9% respectively. There was no much difference between growth of boys and girls. The rising trend of percentage of children who had low weight and height for age was found as they became older. To get a better growth and a better quality of life, the pretransfusion haemoglobin level should be maintained at >6g/dl (probably 6-9g/dl).

INTRODUCTION

Among the inherited disorders, haemoglobinopathies especially thalassaemias and/or haemoglobin E (HbE) is very common in our country. In general these homozygous beta-thalassaemic children have growth retardation. However, the real cause of growth retardation is still controversial. There are many studies showing that the early hypertransfusion regime can prevent growth retardation as well as hypersplenism in thalassaemic children [1,2,3]. In Myanmar, apart from few clinical and epidemiological studies [4,5,6,7], the growth of our thalassaemic children has not been studied yet. The present work was to study the growth of thalassaemic Myanmar children who were treated with the low transfusion regime.

MATERIALS AND METHODS

This study was conducted in Yangon

Children's Hospital (YCH) during June to November 1990. The details of YCH and its Day-Care-Room (DCR) had been described elsewhere [8].

The available data including sex, age of first transfusion and pretransfusion Hb level of all thalassaemic patients (493) both missed and current cases who had registered at DCR were collected from the register book. For 132 current cases apart from above variables, the height (in inches) and weight (in kg) were measured using Tanaka, Model D-20-1 scale, which was available at DCR. The size of liver was measured at right mid clavicular line and the spleen was measured at left anterior axillary line below the costal margin.

Out of 132 current cases, only 118 cases (male 59 and female 59, age 8/12-12 yrs) were included in the analysis as the remainders had incom-

plete data. The height of children in inches was converted into centimeters. Then their weight and height for age were determined using the NCHS standards [9].

RESULTS

The age of patients at first visit to DCR for transfusion ranged from <1-12 years with the median of 6 years. The pretransfusion haemoglobin (Hb) levels of them were 3.0-8.2 g/dl (median 5.0g/dl). All of the patients had moderate to marked enlargement of liver (range 1-18 cm, median 5.5 cm below the right costal margin) and spleen (range 2-16 cm, median 5.5 cm below the left costal margin).

Table 1 shows the distribution of growth indicators of all ages and sex combined. All patients examined had below median -2SD for weight for age (68.6%) and height for age (72.9%). There was no much difference between the growth of boys and girls (Table 2). The distribution

Table 1. Distribution of growth indicators among thalassaemic children, Yangon Children's Hospital, 1990

Nutrition level	Indicators	
	Weight for age	Height for age
No. examined	118	118
No. below median -2SD	81	86
% below median -2SD	68.6	72.9

of low weight and height for age by age classes are shown in Table 3. There was a rising trend of the percentage of children who had low weight and height for age as they became older.

Table 2. Distribution of growth indicators among thalassaemic children by sex, Yangon Children's Hospital, 1990

Nutrition level	Boys		Girls	
	Height/ Age	Height/ Age	Height/ Age	Height/ Age
No. examined	59	59	59	59
No. below median -2SD	43	40	38	46
% below median -2SD	72.9	66.1	64.4	78.0

Table 3. Distribution of low levels of weight/height for age among thalassaemic children by age groups, Yangon Children's Hospital, 1990

Nutrition level	0-4 yrs		5-9 yrs		>10 yrs	
	Ht	Wt	Ht	Wt	Ht	Wt
No. examined	29	29	61	61	28	28
No. below median -2SD	15	17	47	40	24	24
% below median -2SD	51.7	58.6	77.0	65.6	85.7	85.7

DISCUSSION

In the present study, the age of first visit to DCR among the patients were varied widely, but the majority were in older age group. This finding together with the presence of thalassaemia intermedia reported by Aung Than Batu et al. [7], indicated the existence of both Beta⁺ and Beta⁰ thalassaemia gene in our country.

In thalassaemia major, blood transfusion is the main tool for correcting

severe anaemia, as well as for preventing the development of severe secondary characteristics of disease, such as massive hepatosplenomegaly, skeletal deformity and short stature [10]. If transfusion therapy was given to maintain the Hb level consistently above 7.5g/dl, the growth could be improved markedly and the development of serious complications could be delayed [1]. However, in our country, because of the unaffordability of iron chelating therapy, blood transfusion was usually given when anaemia became very marked or clinical conditions impaired. Hence, most of our thalassaemic children had moderate to marked hepatosplenomegaly. Besides, there were also severe bone changes with resultant typical thalassaemic faces.

Regarding the growth and development, the observations from the present study indicated that the thalassaemic children of all age groups had growth retardation in both height and weight. The percentage of children whose weight and height were below median -2SD increased as their age became older. Boys and girls had the same distribution of growth indicator.

The effect of high transfusion regimes on growth is still controversial. Some workers did not find any significant beneficial effect of hypertransfusion on growth [11,12,13]. However, others have shown that high transfusion even with transfusion iron overload gives beneficial effects not only on the growth rate but also produces reduction in heart, spleen and liver size and improves the general health and physical activity of the children [2,14]. Besides, severe anaemia which causes tissue hypoxia may be one of the important factors in producing tissue damage as well as influencing the effect of iron on the myocardium [2]. Therefore, although low transfusion regimen is better for our country where iron chelating therapy is not affordable, in order to

get a better quality of life, the pretransfusion Hb level should be maintained at a moderate level (between 6-9 g/dl) instead of <6 g/dl which is very low.

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Is oral rice-based electrolyte solution superior to glucose electrolyte solution in acute childhood diarrhoea?

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In a controlled trial of oral rehydration therapy, the efficacy and safety of rice-based electrolyte solution was compared with standard WHO oral rehydration solution. Ninety eight male children aged 4 months to 10 years presenting with watery diarrhoea of less than 72 hours prior to hospitalization were studied. 48 were treated with standard WHO oral rehydration solution (ORS) and 50 patients with an improved formulation which contained 100 gm of rice (instead of glucose) with 25 mg of amylase in addition to electrolytes. The clinical characteristics of the two groups on admission were comparable. Although there was a consistent trend towards reduction in stool volume and better gain in body weight in children with cholera treated with rice-based ORS, significant difference was noted only in 6-12 hour stool output and in the first 6 hour weight gain. Stool volume was significantly reduced during the 24 to 48 hour period in all diarrhoeal patients receiving rice-based ORS. Thus, amylyte digested rice-based ORS is as effective as the Standard WHO ORS and have some therapeutic advantage in the treatment of acute childhood diarrhoea.

INTRODUCTION

Glucose-based oral rehydration solutions have provided a simple and successful means of treating or preventing dehydration due to acute diarrhoea in infants and children. Although glucose-based solution stimulate the intestinal absorption of fluid and electrolytes from isotonic luminal contents, they do not aid in the reabsorption of fluid secreted by the

intestine [1]. Thus, these solutions do not lessen the volume, frequency, or duration of diarrhoea [2].

Therefore, search for a more efficient, less costly, easily available and accessible ORT continued and resulted in the development of cereal or food-based oral rehydration therapy.

Rice on hydrolysis yields glucose, amino acids, and oligopeptides which would enhance sodium absorption

through an independent carrier system. The use of digestible food polymers made up of glucose (starch) and amino acid polymers (proteins) in oral replacement solution offers the possibility of increasing the amount of glucose in the ORS formulation without increasing its osmolality. The primary objective of the present study was to compare the effect of amylase digested rice-based ORS versus glucose-based ORS on total stool output, duration of diarrhoea, and weight changes from admission. A randomized controlled clinical trial was carried out comparing an amylyte digested rice-based ORS with the standard WHO ORS in the treatment of dehydrated children with acute diarrhoea.

PATIENTS AND METHODS

Study area and population

One hundred and four male children aged 4 months to 10 years admitted during the monsoon months of 1992 to 1993 at the North Okkalapa General Hospital, Infectious Diseases Hospital and Yangon Children's Hospital were studied. All had acute watery diarrhoea of less than three days' duration with dehydration. Children with coexisting systemic illness like pneumonia, sepsis etc, clinical evidence of severe malnutrition or shock, children who had received antibiotics prior to hospitalization, previous diarrhoea within 2 weeks before the present episode, presence of blood in the stool before admission, were not included in the study.

Relevant history taking and physical examination were carried out on all patients. Initial body weight were taken on admission before commencing rehydration therapy. Informed consent was obtained in every case. With the use of random permuted block of variable length, patients were randomly assigned to receive either the improved

ORS or the standard WHO ORS for rehydration and fluid maintenance. The standard WHO ORS formulation contains sodium chloride 3.5 gm, potassium chloride 1.5 gm, sodium citrate 2.9 gm and glucose 20 gm. The improved ORS contains sodium chloride 1.5 gm, potassium chloride 600 mg, calcium chloride 150 mg and amylase 25 mg. One packet of amylyte ORS added to 100 gm of rice as it is becoming cooked will provide about 220-250 ml of a clear, pleasant tasting ORS. Amylase-digested rice-based ORS containing short polymers of glucose and electrolytes has a higher nutritional and caloric content 42 Kcal/100 ml compared to WHO ORS 8 Kcal/100 ml.

Blood samples for hematocrit, specific gravity, electrolytes and glucose estimation were taken on admission, 6 hr, 12 hr and every other day.

During the initial rehydration phase in the first 4-6 hrs each child was given assigned ORS at a rate of 20 ml/kg/hr to correct dehydration; no other food or fluid were given. This regimen was maintained till clinical signs of dehydration were ameliorated. Thereafter the children were put on maintenance rehydration therapy in which on going stool losses were replaced with ORS on a volume to volume basis every four hours until diarrhoea ceases. Breast feeding, free water and other food were permitted during the maintenance phase. The total intake (fluid and meals) and the urine and stool outputs were recorded. Stool pH and reducing substances after acid hydrolysis and stool examination for ova and parasites were performed. Stool swabs were taken for culture of *V. cholerae* and examined for rotavirus by ELISA.

Body weights were recorded every six hours in the first 24 hours and every 24 hours thereafter. The clinical assessment of patients' response to therapy was repeated regu-

larly until cessation of diarrhoea. Patients were discharged from the study if no diarrhoea occurred for 12 hours or if they were passing formed stools. On admission, stool swabs were taken for culture of Vibrio cholerae.

The study was approved by the medical ethics committee of the Department of Medical Research.

Sample size determination

On the basis of previous trial data, we estimated a pretrial sample size of 50 patients per group as being sufficient to detect a 30% reduction in both the total stool output and the duration of diarrhoea between the two treatment groups, with a power of 80% and a significance level of 0.05.

Significance testing

The data were analysed by computer using the SPSS/pc +V2.0 (Chicago, Illinois, USA). Significant test used were X^2 analysis with Yate's correction, two-tailed t-test and Mann-Whitney U non parametric test as appropriate.

RESULTS

One hundred and four patients were enrolled in the study. Two patients in WHO ORS treated group who had treatment failure, passing liquid stool of more than 5 ml/kg/hr, two children requiring antibiotics therapy for complicating illness and another two children who after randomization did not pass any diarrhoeal stool were excluded from the final analysis. Thus 98 patients were eligible for analysis; 48 receiving WHO ORS and 50 the amolyte ORS.

Admission characteristics of the two treatment groups were comparable. Table 1 shows the comparison of

main outcome variables in all diarrhoea cases in two treatment groups. Significant reduction of stool output in the rice-based treated group was noted on the second day of diarrhoea. Although there was weight gain at the different study period and on discharge in the study group together with reduction in total mean stool output and duration of diarrhoea in rice-based ORS receiving group, the differences were not significant.

Patients in the two treatment groups were stratified into those in whom V. cholerae was detected or not in the stool. In non cholera group mean stool output was significantly reduced on second day in rice-based ORS receiving group. Lesser total stool output and duration of diarrhoea with better weight gain on discharge had been noted in the study group, the differences were statistically not significant. On the other hand amount of vomitus was significantly greater in rice-based ORS treated group (Table 2).

Among cholera patients there was consistent trend towards better weight gain in the rice-based ORS treated group but significant difference was noted in the initial rehydration phase (Fig. 1). Similarly, reduction in stool output was consistent throughout the study periods with the significant difference being 6-12 hour period (Fig. 2).

Rice based ORS was accepted well and none of the patients developed hypernatremia, hyponatremia or other electrolyte disturbances or carbohydrate intolerance.

DISCUSSION

The results of a meta-analysis of 13 randomised clinical trials comparing the effects of WHO glucose-based ORS and rice-based ORS had been

Table 1. Comparison of main outcome variables in the two treatment groups

Outcome variables	Treatment		95% confidence interval	
	WHO ORS n = 48	Rice-based ORS n = 50		
Mean stool output (gm/kg admission weight)				
0 - 6 hr	23.19 ± 37.34	18.97 ± 20.44	-7.874	16.322
6 - 12 hr	16.68 ± 29.97	12.22 ± 15.97	-5.188	14.108
12 - 24 hr	20.86 ± 29.45	51.32 ± 239.19	-100.03	39.105
*24 - 48 hr	20.77 ± 26.18	10.53 ± 15.90	1.755	18.731
0 hr to recovery	90.53 ± 110.71	59.97 ± 54	-4.42	65.532
Mean ORS intake (ml/kg admission weight)				
0 - 24 hr	192.50 ± 104.35	185.49 ± 63.25	-27.68	41.72
24 - 48 hr	68.86 ± 78.35	47.73 ± 44.04	-4.41	46.68
0 hr to recovery	285.65 ± 179.88	239.68 ± 98.10	-12.85	103.59
Mean weight gain (% of admission weight)				
0 - 6 hr	1.31 ± 5.17	2.46 ± 4.27	-3.072	0.768
6 - 12 hr	4.99 ± 20.40	3.18 ± 4.54	-4.11	7.726
12 - 24 hr	3.51 ± 6.87	4.09 ± 4.80	-2.98	1.811
24 - 48 hr	2.61 ± 9.12	6.10 ± 6.08	-8.096	1.132
wt gain on D/C (%)	0.66 ± 16.61	5.04 ± 6.78	-9.47	0.71
Duration of diarrhoea after admission	34.75 ± 18.82	30.87 ± 14.03	-2.81	10.57
Vomitus* (ml/kg)	1.70 ± 4.45	7.04 ± 13.85	-9.527	-1.139
Urine output (ml/kg)	74.90 ± 80.74	64.64 ± 43.10	-15.74	36.27
Frequency of diarrhoea	11.35 ± 7.1	4.82 ± 5.15		

demonstrated that the beneficial effects of rice-based ORS on the rate of stool output was significantly greater in patients with cholera. However, for acute non-cholera diarrhoea in infants and young children, the effect was substantially smaller.

In our study it has been shown that the cholera patients treated with rice-based ORS had significant weight gain and reduced stool output during the first 12 hours together with consistent trends towards lower stool output and better weight gain during the study period till discharge and lower frequency of diarrhoea.

However, non-cholera patients treated with rice-based ORS had significant reduction of stool output on 2nd day and was not accompanied by greater weight gain in compare with ORS treated groups. The significant amount of vomitus obtained in non-cholera patients receiving rice-based ORS might be the possible cause for no weight gain in these patients.

Our findings support the fact that rice-based ORS has greater beneficial effect on stool output in patients with cholera than in non-cholera, indicating that there was better absorption of fluid and electrolyte

Table 2. Comparison of main outcome variables in non-cholera in the two treatment groups

Outcome variables	Treatment		95% confidence interval	
	WHO ORS n = 48	Rice-based ORS n = 50		
Mean stool output (gm/kg admission weight)				
0 - 6 hr	22.24 ± 41.90	20.20 ± 22.16	-13.58	17.67
6 - 12 hr	13.24 ± 32.14	14.10 ± 17.19	-12.8	11.16
12 - 24 hr	14.44 ± 15.70	62.75 ± 276.02	-144.6	47.99
*24 - 48 hr	20.10 ± 24.80	10.18 ± 14.51	0.33	19.50
0 hr to recovery	78.40 ± 110.96	62.39 ± 55.22	-24.79	56.79
Mean ORS intake (ml/kg admission weight)				
0 - 24 hr	191.61 ± 117.41	183.17 ± 64.93	-35.83	52.71
24 - 48 hr	68.51 ± 79.91	45.61 ± 41.81	-6.82	52.61
0 hr to recovery	284.70 ± 190.92	235.20 ± 97.91	-21.16	120.16
Mean weight gain (% of admission weight)				
0 - 6 hr	1.40 ± 5.74	1.64 ± 3.85	-2.55	2.07
6 - 12 hr	6.68 ± 23.93	2.52 ± 4.55	-3.75	12.08
12 - 24 hr	3.71 ± 7.12	3.63 ± 4.75	-2.76	2.92
24 - 48 hr	2.07 ± 9.69	4.63 ± 5.70	-8.13	3.01
wt gain on D/C (%)	-0.54 ± 19.46	4.49 ± 7.05	-11.80	1.73
Duration of diarrhoea after admission	35.58 ± 20.35	28.87 ± 13.64	-1.41	14.84
Vomitus* (ml/kg)	2.14 ± 5.24	4.92 ± 11.40	-7.11	1.53
Urine output (ml/kg)	78.75 ± 85.01	67.15 ± 45.16	-20.13	43.33
Frequency of diarrhoea	10.76 ± 7.83	8.87 ± 5.48		

for rice-based ORS. It may be due to the fact that in cholera, gut epithelium remains intact and absorption is effective throughout the length of small intestine. This presumably reflects that a greater amount of glucose (and amino acid) is released when rice is fully digested than is present in the WHO ORS. Since the number of patients with cholera in our study was small, this finding should be reviewed in the light of further studies of rice-based ORS.

In conclusion, this study demonstrated that rice-based ORS is as effective as the WHO ORS and has some

therapeutic advantage in the treatment of childhood diarrhoea.

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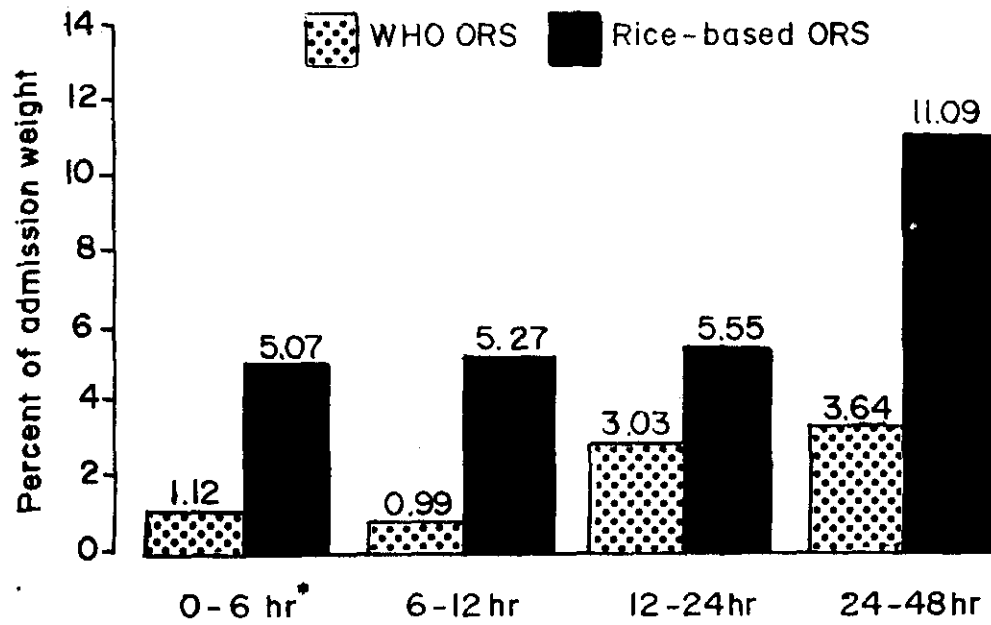


Fig. 1. Percent weight gain in cholera patients
*Significantly different

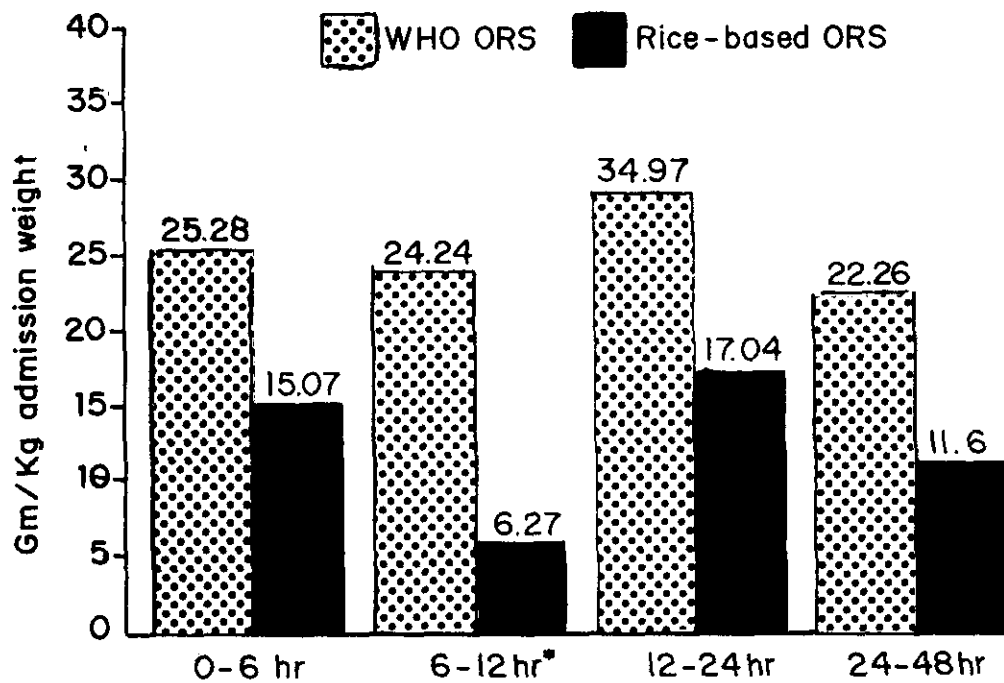


Fig. 2. Stool output in cholera patients
*Significantly different

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**Relationship between serogroups and enterotoxin production
of Escherichia coli from children with diarrhoea**

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It has been reported that the enterotoxigenic Escherichia coli could be serotyped in various countries and some serotypes were restricted to each area. Thus a study was conducted on 2522 Escherichia coli isolates from 501 diarrhoeal cases and 374 control cases from the Intakaw survey. Enterotoxigenic strains were isolated from 91 cases of diarrhoea and from 29 control cases. It was found that the serogroups 0126 and 0114 were associated with heat-stable (ST) and heat-labile (LT) toxin producing isolates respectively. 027 and 06 serogroups showed a correlation with ST alone and STLT producers respectively. Though the other serogroups sought were 01, 026, 0119, 0159, 0127, 0128, 0148 and 0159, it was noted that only 60 percent of the enterotoxigenic E. coli could be serogrouped. The serotyping of flagella was also done and different patterns including H8, H12, H19, H21, H32, H38, H49 and H51 were observed.

INTRODUCTION

Currently, Escherichia coli serotypes and correlation with enterotoxin production had been reported by various workers [1-5]. It seems that diversity exists among strains in geographical areas and since it is important to know the widespread nature of the enterotoxin-producing E. coli strains and their association with diarrhoea, we have taken the opportunity of serotyping and testing for enterotoxins.

MATERIALS AND METHOD

The study sample consists of 330 cases of diarrhoea and 233 cases of control in hot wet season and 171 cases of diarrhoea and 141 cases of control in cool dry seasons and the methods of selection of cases and control were described in our previous study [6]. The subjects comprise of children of less than 5 years of age. The standard laboratory procedures were used for bacteriological methods [6]. From this survey a total of

2522 *Escherichia* isolates were tested for enterotoxin production and then serotyping was done as described by Ewing 1986 [7].

RESULTS

Enterotoxigenic *Escherichia coli* isolates from acute diarrhoea and control children in Intakaw are shown in Table 1. It was observed that 75

Table 1. Enterotoxigenic *Escherichia coli* isolated from acute diarrhoea and control children in Intakaw

	Hot wet season		Cool dry season	
	Diarrhoea	Control	Diarrhoea	Control
Cases	330	233	171	141
ST	35(10.6)	11(4.7)	9(5.3)	2(1.4)
LT	26(7.9)	8(3.4)	2(1.2)	2(1.4)
STLT	14(4.2)	4(1.4)	5(2.9)	2(1.4)
EPEC	75(22.7)	23(9.5)	16(9.4)	6(4.3)

Figures in parentheses denote percentages

cases (22.7 percent) and 16 cases (9.4 percent) were isolated as enterotoxigenic *E. coli* from diarrhoea cases from hot wet and cool dry seasons respectively. From the diarrhoea cases it was found that during the hot wet season isolation rate for heat stable *E. coli* (ST) was 10.6 percent (35 cases), for heat labile *E. coli* (LT) 7.9 percent (26 cases) and *E. coli* (STLT) 4.2 percent (14 cases) respectively. During the cool dry season the isolation rate for ST was 5.3 percent (9 cases), for LT was 1.2 percent (2 cases) and for STLT 2.9 percent (5 cases) respectively.

Relationship between serogroups and enterotoxin production is shown in Table 2. It was observed that 11 percent of diarrhoea and 14 percent of control which produced LT alone were correlated well with the 0114 serogroup. Also, 15 percent of diarrhoea cases and 21 percent of control

which produce ST alone were correlated with 0126 serogroup. Also 6 percent and 4 percent of diarrhoea cases of *E. coli* which produced ST alone were shown to have 027 and 0148 serogroups respectively. 9 percent of *E. coli* from diarrhoea cases which produce STLT were of 06 serogroups. It was also noted that 72 out of 120 (60 percent) cases of either diarrhoea or control could be serogrouped and found to be 60 percent correlated with EPEC and serogroup. Thus there is a relationship between the serogroup and enterotoxigenicity.

Moreover, the 'H' serotypes were also investigated by tube agglutination test. It was demonstrated that out of 10 cases of 0114 serogroup, H serotypes showed different pattern of H21, H32, H38, H49, H- and HUT. From 20 cases of 0126 serogroup the H serotypes sought were H8, H12, H19, H51, H- and HUT.

DISCUSSION

Merson et al. [8] used antisera of 110 serogroups and were able to serotype 64 percent of the EPEC isolated in Bangladesh. Changes in serotypes of Enterotoxigenic *E. coli* in Dhaka over time and usefulness of polyvalent antisera has been reported since 1983 [9]. Rowe et al. also reported that they were able to serotype 60 percent of EPEC from various Asian countries. 06, 08, 025, 027, 063, 078, 0115, 0148 and 0159 were the most common serotypes in their studies and suggested that the pattern of distribution of O serogroups of EPEC may be more or less the same throughout the world and thus the serotyping of EPEC by using antisera may be useful in initial screening of EPEC [10].

From various studies it was noted that 46 to 66 percent of the EPEC were able to be serotyped [8,9,11]. Study by Iyer [12] and Goyal [13]

Table 2. Relationship between *E. coli* serogroups and enterotoxin production

Serogroups	Diarrhoea (91 cases)				Control (29 cases)			
	ST	LT	STLT	ETEC	ST	LT	STLT	ETEC
0114	0	10	0	10	0	4	0	4
0119	0	0	0	0	1	0	0	1
0126	14	0	0	14	6	0	0	6
0127	1	0	0	1	0	0	0	0
0112	0	0	0	0	1	0	0	1
0136	1	0	0	1	0	0	0	0
06	1	0	8	9	0	1	1	1
08	2	0	0	2	0	0	3	3
025	0	0	1	1	0	0	0	0
027	5	0	0	5	0	0	0	0
078	0	0	0	0	1	0	0	1
0148	4	0	0	4	1	0	0	1
0159	0	1	1	2	0	0	0	0
06 + 0126	0	0	1	1	0	0	0	0
06 + 0128	0	0	1	1	0	0	0	0
027 + 0159	1	0	0	1	0	0	0	0
026 + 078	1	0	0	1	0	0	0	0
Knowns	30	12	12	54	10	4	4	18
Untypable	14	16	7	37	3	6	2	11
Total	44	28	19	91	13	10	6	29

ST = Heat-stable toxin

LT = Heat-labile toxin

ETEC = Enterotoxigenic *E. coli*

claimed that ETEC were not restricted to a few 'O' types. In their study 60 percent of ETEC could be serotyped. 0126 was most frequently associated with ST alone and 0114 showed a relationship with LT alone and it was found to be different from the study of Merson or Rowe [8,10]. The most common serogroups reported by Merson was 078 and 0115 whereas Rowe reported that 0148, 06, 025 were the most common serogroups. In our study, besides 0114 and 0126, 027 showed a relationship with ST alone and 06 with STLT. The other frequent associated serogroups found were 08, 0148, 0159, 0127 and 01.

Orskov and Orskov [14] also claimed that the enterotoxin products confined to some of the serogroups. In this

study ST were restricted to 0126, 0127, 0136, 06, 08, 027, 0148. LT were restricted to 0114 and 0159, STLT were restricted to 06, 025 and 0158. The serogroups 0126 and 0114 were most associated with heat stable (ST) and heat labile (LT) producers respectively.

Soe Thein [15] studied the serogroups of *E. coli* in diarrhoea children and reported that 0128, 0119, 0125, 0126, 026, 055, 0127 and 0111 were common and played a significant role in diarrhoea. In this study some of the O serogroups showed a significant role in the diarrhoea in compare to controls. Those include 06, 08, 027, 0114, 0126, 0148 and 0159. It was also observed that 078, 0128 and 0167 were not restricted to toxin producers. In 0126 serotypes H12

was encountered in cases of ST alone, which was similar to Rowe's findings.

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Survival of Vibrio cholerae and Vibrio parahaemolyticus in natural waters of Yangon

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Laboratory studies were conducted to examine the survival of V. cholerae and V. Parahaemolyticus in untreated and autoclaved samples of natural waters collected from different sources. Physico-chemical parameters of the natural waters were also examined as the turbidity, pH, hardness, dissolved solids, oxidizable organic matter, the chloride content, etc. The survival rates were found to vary from 2 to 7 days for V. cholerae and from 30 minutes to 9 days for V. parahaemolyticus in raw natural waters. Both organisms survived for prolonged period in river water that had been autoclaved.

INTRODUCTION

Diarrhoeal diseases are major health problems in Myanmar. As water has been considered to be the main vehicle of infection in cholera, gastroenteritis and other diarrhoeal diseases, the survival time of vibrios in natural waters is of great importance from the epidemiological point of view.

Vibrio parahaemolyticus is one of the most recent organisms to be associated with gastrointestinal food poisoning. The pathogen has been isolated from seafood, sea water, sea mud and also from freshwater, fish, crabs and shrimps in different parts of the world. The frequency of isolation of the organism from diarrhoeal cases in different countries varies widely. In Yangon there was a report on the isolation of that organism in about 3.4% of clinical cases of diarrhoea in 1979 [1].

The viability of V. cholerae in water has been studied by other workers but the survival rate widely varied [2,3,4,5]. Furthermore, several species of vibrios have been isolated from

the natural waters of Yangon [6] and the present study was done to determine the survival of two species of pathogenic vibrios in these waters and their current physicochemical characteristics.

MATERIALS AND METHODS

Collection of samples

The materials selected for the study included (a) pond water from a pond in front of Department of Medical Research (b) creek water from Ngamoyeik creek (c) lake water from Kandawgyi lake (d) river water from Hlaing river (e) superficial well water from a well in Mayangone Township, and (f) water from a deep well in Mingalar Taungnyunt Township. People usually use the first four for bathing and washing and the latter two are used for domestic purposes as well as for drinking. Four litres of water from each of the above sources were collected in sterile containers employing the standard method of collection [7]. The samples were immediately transported to the

laboratory on ice and processed within one hour after arrival. Two strains of vibrios were used as contaminants in this study. V. cholerae 01 (Inaba, El Tor strain) and V. parahaemolyticus (Kanagawa positive strain). They were maintained on trypticase soy agar medium at room temperature and subcultured at weekly intervals.

Physicochemical examination

Physicochemical analyses of these natural water samples were made for appearance, colour, smell, sediment, nitrites, total solids, chlorides, hardness, iron, dissolved oxygen, total alkalinity and pH in National Health Laboratory, Yangon [8,9].

Experiment on survival time

Eighteen hour peptone-water cultures of V. cholerae and V. parahaemolyticus kept overnight at 37°C were used as inoculums. 100 ml of water sample from each source was autoclaved at 121°C for 15 minutes. 100 ml each of raw and autoclaved water samples were contaminated with each of the above vibrio spp. to a final concentration of 10⁵ colony forming units per ml. The contaminated samples were then incubated at room temperature (24°C) and viable counts of vibrios were determined by drop plate method of Miles & Misra [10] using Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar at one hour intervals for 4 hours on the first day and one time daily up to 7 days. Then the examination was continued at weekly intervals until the viable counts were negative.

RESULTS

Fig. 1 and 2 show the survival times of V. cholerae and V. parahaemolyticus in various samples of natural waters. In untreated samples V.

cholerae survived for 2 days in pond water, 4 days in deep well water, 5 days in lake water, 6 days in river water, 7 days in creek and superficial well water. In the autoclaved water samples it survived for 17 days in creek water, 23 days in deep well water, 38 days in superficial well water, 37 days in lake water, 49 days in pond water and 158 days in river water.

The viability of V. parahaemolyticus in raw water samples were observed as 30 minutes in pond water, 3 hours in deep well water, 1 to 3 days in lake, creek and superficial well water and up to 9 days in river water. In autoclaved samples it survived for 1 to 4 hours in pond, lake, creek and deep well water. The survival time in shallow well water was 1 day and prolonged survival was observed in river water as 20 days.

Chemical analyses of natural water samples (Table 1) show that there were excessive amount of total solids, chlorides, hardness and organic matter in creek and river waters. Iron was generally present in all samples and excessive in river water. The effect of autoclaving on physicochemical properties of water was also examined and it was observed that the pH increased from 6.4-8.2 in raw water to 7.2-9.2 after autoclaving. The chlorides and total solids contents increased only in river water due to autoclaving.

DISCUSSION

The viability of V. cholerae in untreated samples from different sources was found to vary from 2 days in pond water to 7 days in creek and shallow well water. The survival time considerably increased in samples that had been autoclaved to eliminate competing microorganisms for nutrients. The longest survival time for 158 days was observed in autoclaved

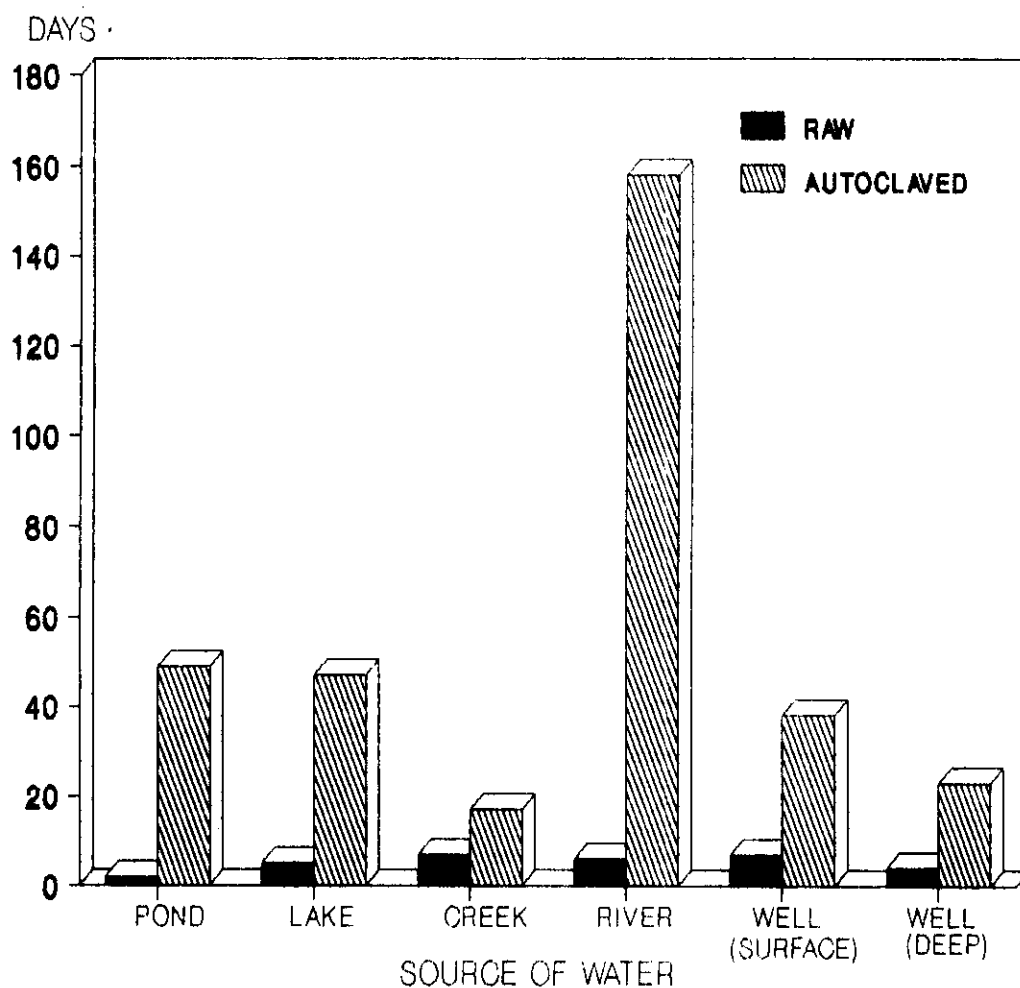


Fig. 1. Survival of *V. cholerae* in natural waters (raw and autoclaved)

river water. *V. cholerae* survived well in water samples from creek, river and superficial well which were found to contain abundance of total solids and chlorides. Autoclaving turned the pH of water samples to alkaline side which is favourable for the survival of vibrios.

It is seen that with the exception of river water *V. parahaemolyticus* did not survive long in natural water samples, whether raw or treated. It was observed that water and sediments offer a rather limited ecological niche for *V. parahaemolyticus* [11,12]. Its survival for a longer period in

river water may be due to the presence of requisite conditions in that water as far as chlorides, organic matter and total solids contents were concerned.

From our study it is observed that both *V. cholerae* and *V. parahaemolyticus* survived a considerable length of time especially in creek, river and shallow well water and it indicates that such waters if contaminated with these organisms can transmit the infection and contribute health hazard to the public for a considerable period.

Table 1. Physicochemical examination of natural water samples

Physicochemical parameters	Pond water		Lake water		Creek water		River water		Superficial well water		Deep well water	
	Raw	Autocl.	Raw	Autocl.	Raw	Autocl.	Raw	Autocl.	Raw	Autocl.	Raw	Autocl.
Appearance	ST	C	ST	C	ST	ST	T	T	C	C	C	C
Colour as unit	12	12	12	12	12	12	15	12	10	10	10	10
Smell	nil	nil	nil	nil	nil	nil	mud	mud	nil	nil	nil	nil
Sediment	mod	sl	sl	sl	sl	sl	mod	mod	sl	sl	sl	sl
pH value	8.2	9.0	7.2	9.2	7.4	8.2	7.1	8.6	6.4	7.2	7.0	7.6
Total solids, mg/l	280	218	196	200	2460	2458	4380	4640	380	390	70	88
Chlorides, mg/l as chlorine	27	27	17	17	1020	1080	2280	2510	109	113	12	12
Total hardness, mg/l as CaCO_3	84	84	58	62	860	860	360	380	86	86	20	22
Oxygen absorbed, mg/l as O_2	2.66	2.66	3.33	3.00	1.00	0.33	1.00	1.00	0.66	0.66	1.00	0.33
Total alkalinity, mg/l, CaCO_3	68	73	59	60	64	68	51	51	50	50	28	32
Iron, mg/l as Fe	0.92	0.60	0.66	0.46	0.46	0.27	3.01	1.31	0.13	0.13	0.86	0.86
Nitrites, mg/l as nitrogen	nil	nil	nil	nil	nil	0.001	nil	0.004	0.020	0.006	nil	nil

Autocl. = Autoclaved, ST = Slightly turbid, T = Turbid, C = Clear, mod = moderate, sl = slight, mud = muddy

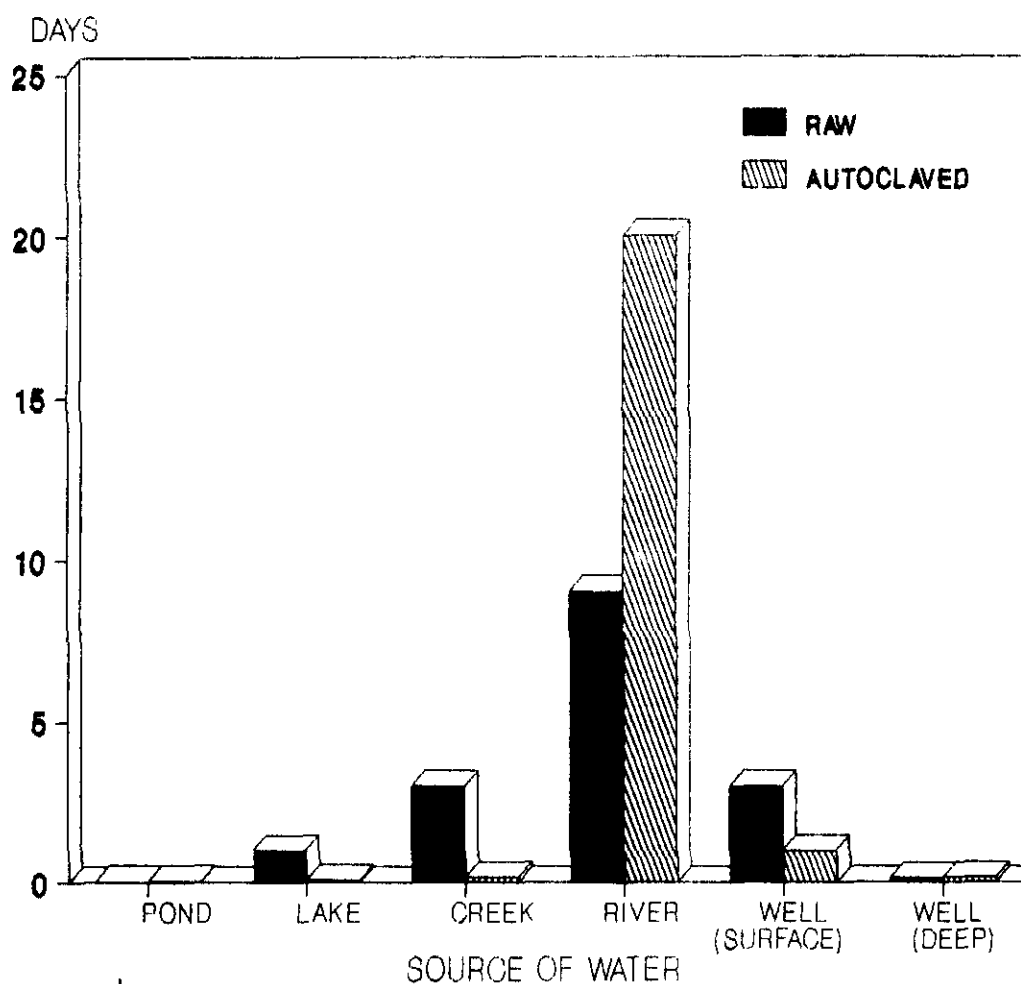


Fig. 2. Survival of V. parahaemolyticus in natural waters (raw and autoclaved)

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SHORT REPORT

Small intestinal permeability in apparently healthy Myanmar adults

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The jejunal mucosa of people living in tropical countries show morphological abnormalities often with xylose malabsorption. The functional significance of these morphological changes is uncertain although many studies in these countries have demonstrated that abnormal D-xylose absorption is very common in these people [1]. Menzies *et al.* [2] have reported a method for assessing small intestinal integrity by measuring passive permeability of the small intestine to L-rhamnose (a monosaccharide, M.W 160) and lactulose (a disaccharide, M.W 340), both sugars being quantitatively excreted in the urine following their unmediated intestinal absorption. The influence of extraneous factors such as age, renal function, gastric emptying and intestinal bacterial overgrowth are minimized by expressing the results as a excretion ratio (lactulose/rhamnose excretion ratio).

The present study was carried out to determine small intestinal permeability in a group of apparently healthy Myanmar subjects to see if there is evidence of intestinal dysfunction and tropical enteropathy in Myanmar subjects. Twenty five apparently healthy Myanmar subjects of both sex (age 21-25 years) without evidence of gastrointestinal or systemic disease took part in the present study. After overnight fast, the subjects drank a 100 ml test solution containing 5g of lactulose (7.5 ml of Duphalac Syrup) and 1g of L-rhamnose. All the urine passed in the subsequent five hours was collected in a plastic bottle containing merthiolate (100

mg/dl) as preservative. The volume of urine was recorded and an aliquot of it was stored at -10°C until analysis. Thin layer chromatography was used to determine the urinary sugar content [2].

The mean ($\pm$ SE) five hour urine excretion of lactulose and L-rhamnose were $0.292 \pm 0.038\%$ (range: 0.100-0.850%) and $4.941 \pm 0.527\%$ (range: 1.470-12.66%) respectively. The mean ($\pm$ SE) five hour lactulose/rhamnose ratio was 0.067 ± 0.008 (range: 0.010-0.170).

As a group, Myanmar subjects demonstrated higher lactulose/rhamnose sugar excretion ratios than English subjects [3]. This excretion ratio is a sensitive indicator of small intestinal integrity and these abnormalities indicate significant differences between the small intestine of healthy Myanmar and that of English subjects. The abnormal lactulose/rhamnose excretion ratios are the result of increased absorption of lactulose or decreased absorption of rhamnose or both. In the present study, the percent of oral dose of lactulose excreted by our subjects was found to be parallel to that of English subjects (0.29% vs 0.30%) [4] suggesting that Myanmar subjects may have normal permeability of mucosa for this sugar. The reduction of rhamnose absorption in our subjects may suggest the presence of a diminished absorptive area of intestine as compared with the English subjects (4.94% vs 11.64%) [4] which will accord with the finding that throughout most of

Asia, Africa, Central and South America, their normal jejunal mucosa are most likely to be composed of leaf and convoluted shaped villi with minor degree of atrophy as compared to the mixtures of leaf and finger shaped villi found in the adult western population [5]. The increased sugar ratios found in Myanmar subjects have been supported by another finding that people living in tropical countries have higher lactulose/rhamnose excretion ratios than the western people, but these ratios are comparable among the people living in tropical countries (Table 1). The aetiological

Table 1. Geographic variation in intestinal permeability (in ascending magnitude)

Asymptomatic indigenous adults	Number of subjects	Lact/rham ratio Mean (SEM)
United Kingdom	58	0.026 (0.001)
Botswana	16	0.034 (0.003)
Greece	32	0.037 (0.002)
Italy	18	0.038 (0.004)
South India	21	0.052 (0.008)
Jamaica	14	0.054 (0.009)
Thailand	25	0.055 (0.007)
The Cameroons	17	0.064 (0.010)
Indonesia	25	0.067 (0.007)
Gambia	26	0.096 (0.019)
New Guinea	17	0.156 (0.024)
Myanmar	25	0.067 (0.008)

Source: Jenkins *et al.*, 1989 [3]

factors responsible for this difference are not certain, but are largely environmental and may include nutritional status, genetic factors, luminal parasites, bacterial and viral infection, diet and geographic regions [3].

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SHORT REPORT

Does Russell's viper venom interact with human complement system?

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The complement system is a series of serum proteins which interacts enzymatically to mediate both the beneficial and damaging effects of antibody or antigen-antibody complexes. Activation of the system may occur via either the classical pathway or the alternate pathway with resultant involvement of a terminal complement complex. Studies have demonstrated that snake venom from certain species shown complement activating effects. The detailed study by Muller Eberhard *et al.* [1] showed the complement consuming properties of Egyptian cobra (i.e. *Naja naja*) venom. Evidence for complement activation via the alternative pathway has been observed following bites by Russell's viper, Saw scaled viper and the Boom slang and has been attributed to a non immune mechanism [2,3]. Whether Russell's viper venom (Myanmar) has an interaction with human complement system was studied, *in vivo*, on a total of 18 proven Russell's viper bite patients with serial measurement of complement proteins as well as *in vitro* experiments (i.e. functional haemolytic assays and quantitative measurement of C1q, C3 and C4).

A transient fall in C3 level was observed at 12-24 hour of admission (i.e. 14-30 hour) after the bite, particularly in patients with disseminated intravascular coagulation (Figure 1). A gradual rise was observed in patients with non DIC and those who recovered from systemic envenomation. But Russell's viper venom in *in vitro*

studies showed no complement inactivating activity and no decrease in individual complement proteins.

Although certain snake venoms have been shown to possess anticomplementary activity, we could not demonstrate significant interaction between Russell's viper venom and human complement system. The transient fall in C3 in patients with severe envenomation may be related to changes in vascular and subsequent leakage of plasma proteins. It is reported that various proteins such as trypsin, plasmin [4] and lysosomal derivative [5] are known to have complement consuming activity. Therefore, the other possibility may be due to the release of proteolytic enzymes from various tissues as a result of non immunological events such as ischaemia, necrosis and disseminated intravascular coagulation which result cleavage of complement components similar to that observed with immune activation. This may lower complement levels and generate fragments in a manner indistinguishable from that seen with antibody related reactions. These non immunological modes of activation might be responsible for the changes seen in some patients after snake bite.

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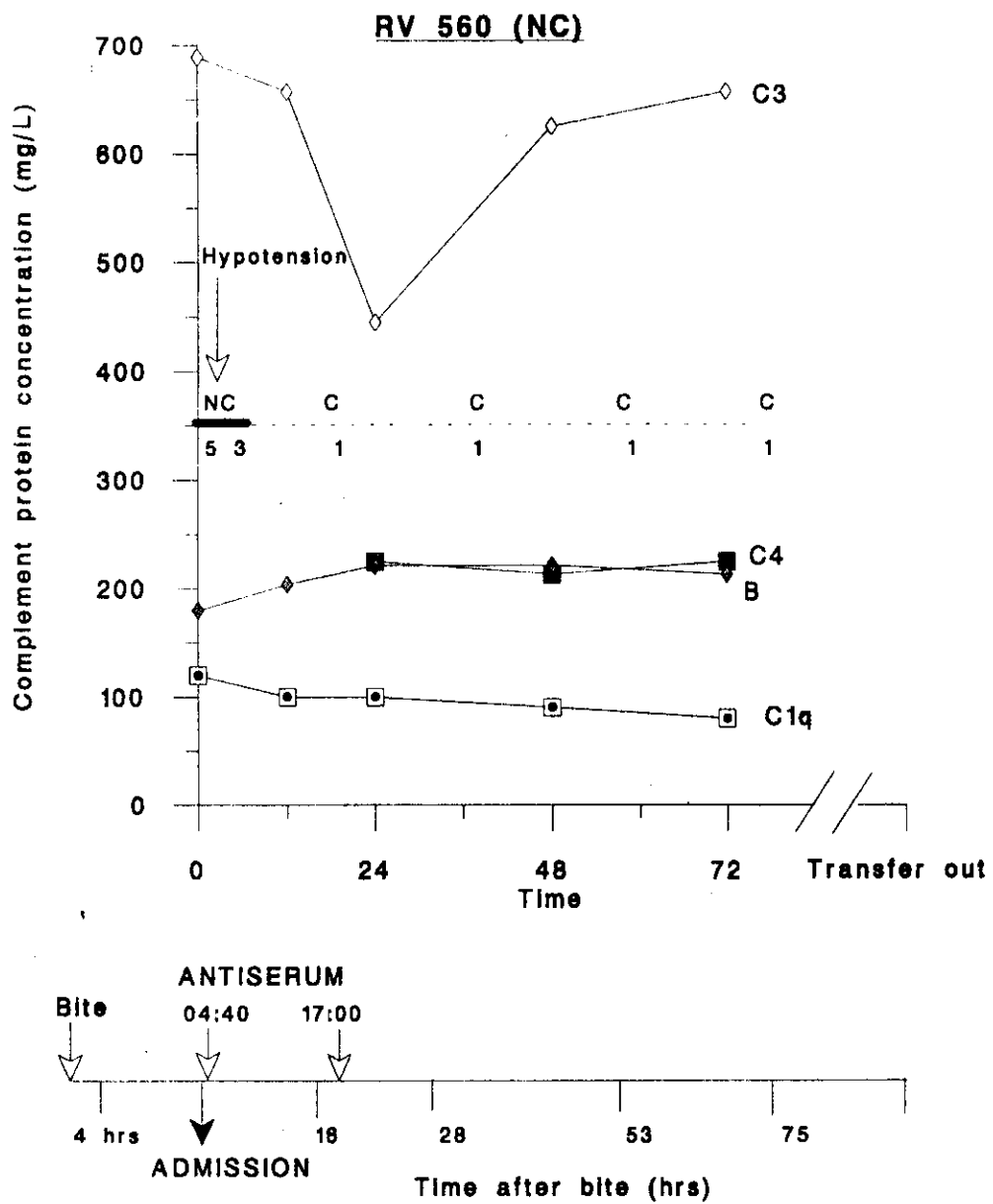


Fig. 1. Complement changes in Russell's viper bite patient (RV 560) whose blood was unclottable on admission.

Clot quality: 1 = normal and 5 = non-clotting (NC). Normal range for complement proteins: C3 800-1536 mg/L; C4 142-447 mg/L; C1q 124-208 mg/L; Factor B 200 mg/L.

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