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Socio-economic and health consequences among HIV/AIDS affected families and orphans in Hlinethaya Township

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With the objectives of identifying the socio-economic and health consequences among HIV/AIDS orphans, a community-based study was conducted employing qualitative research methods in 2009. In-depth interviews with 16 parents/guardians and 18 key informant interviews with the basic health staff, community volunteers and responsible persons from an international non-governmental organization were carried out in Hlinethaya Township, Yangon. There were 41 orphans comprised of 18 double orphans, 21 paternal orphans and 2 maternal orphans. Four orphans and eight guardians were HIV positive. Social consequences included family dispersion, effects on education and stigma/discrimination. Family dispersion was seen in three out of 18 families. There was no orphan currently under care of or stayed with non-relative guardians. About half of school going age orphans dropped out from the school because of economic hardship. Some older children had to work to help the single parents and younger siblings. Discrimination was uncommon among the extended family members. However, disclosure of HIV status affected the working opportunity of single parents. Although change in family possession was not significant, many families had difficulty in struggling for family's daily expenses along with the loss of the bread winner. Vertical transmission of HIV was seen in 4 children. Malnutrition and TB were common health problems for HIV infected orphans. All affected families have some extent of socio-economic consequences from HIV/AIDS. Sustainability and strengthening of support programs should be strongly considered since availability of these support networks could alleviate the negative consequences.

INTRODUCTION

Globally, HIV pandemic remains a serious challenge to public health and AIDS is the leading cause of death worldwide for people aged 15 to 49 years. It was estimated that there were 33.3 million people living with HIV at the end of 2009. In Asia, this figure increased from 4.2 million in 2001 to 4.9 million in 2009 [1].

Epidemic had left behind 16.6 million AIDS orphans by the end of 2009, defined as those aged under 18 who have lost one or both parents to AIDS [1]. Even with anti-retroviral treatment, it is estimated that the number of orphans will still be over-

whelmingly high by 2015 [2]. Orphans in some sub-Saharan African countries exceed one million, and, in some countries, children who have been orphaned by AIDS comprise half or more of all orphans nationally [3]. Most AIDS orphans who live outside of Africa live in Asia, where the total number of orphans for all reasons exceeds 73 million [4]. Estimated AIDS orphans in Asia were more than 1 million [1]; however, there was no estimation of AIDS orphans in Myanmar.

HIV/AIDS has much direct and indirect impact on children's rights, including psychological impact, limited access to quality education and health services.

Previous studies focusing on HIV/AIDS orphans in China and Africa have highlighted the psychological problems [5, 7] and unmet basic needs like food inadequacy, discontinuation of schooling and inaccessible to health care services [7].

Myanmar is one of the countries afflicted by HIV/AIDS like other developing countries in Southeast Asia. According to NAP, it was estimated that adult HIV prevalence is 0.61% [8]. This study was done as there were limited studies on HIV/AIDS orphans in Myanmar.

Objectives

- To determine the socio-economic consequences faced by HIV/AIDS orphans
- To determine the health consequences faced by HIV/AIDS orphans

MATERIALS AND METHODS

A community-based, cross-sectional study was conducted using qualitative approach: in-depth interview (IDI) and key informant interview (KII). Hlinethaya Township from Yangon Region was randomly selected with a consideration of being a peri-urban township, having some community-based activities focusing on HIV/AIDS.

Parents/guardians of HIV/AIDS orphans, Basic Health Staff, community volunteers and responsible persons from World Vision (WV), an International Non-governmental Organization, were included in the study. The HIV/ AIDS orphans were defined as the orphans under the age of 15 years who lost one or both parents due to AIDS.

Data collection

A total of 16 IDI with the guardians of HIV/AIDS orphans and KII with four Basic Health Staff (BHS), six community volunteers and four responsible persons from WV were carried out. Guardians consisted of fathers, mothers, grandparents, elder siblings and aunts/uncles.

All interviews were conducted by the investigators and well-trained research assistants.

Both tape-recording and note taking were made after getting informed consent.

Data analysis

Transcripts were prepared after the field data collection. The research team reviewed all the transcripts and discussed to clarify the findings. Themes and sub-themes were identified carefully and thematic analysis was performed. ATLAS.ti 5.2 demo version was used for coding and organizing the themes. Findings from IDIs and KIIs were triangulated for validation.

Ethical consideration

The proposal was approved by the Institutional Ethical Review Committee, DMR-LM. Written informed consent was obtained from the participants after thorough explanation about the study. To ensure confidentiality, only code numbers were mentioned on the interview records.

RESULTS

Background characteristics

A total of 18 affected families were included in the study. There were 41 orphans comprising of 18 double orphans, 21 paternal orphans and 2 maternal orphans. Double orphans mean orphans who lost both parents due to HIV/AIDS. Orphans' age ranged from 4 months to 15 years with the mean age of 7.2 years (SD-2.1) while guardians' age ranged from 25 to 66 years with the mean age of 47 years (SD-6.3). Among the orphans, 6 children were under 5 years old.

Four out of 41 orphans and 8 out of 9 single parents were HIV positive. Two children already died from the disease before the study. Most double orphans stayed with their grandparents and aunts/uncles. Majority of the guardians mentioned that they have the responsibility to take care as they are the close relatives.

Social consequences

Social consequences included family dispersion, effects on education and stigma/discrimination.

Family dispersion

Dispersion of family members was seen in three out of 18 families. Most orphans could stay with their siblings and single parent together with the extended family members. Some older siblings got married and younger siblings were looked after by other relatives like grandparents, aunts. There was no orphan currently taken care or stayed with non-relative guardians.

"...we have 5 siblings...I'm the eldest...I got married before my mother died...2nd brother got married and 3rd brother was lost from home after my mum passed away... until now we couldn't find him...4th one is a sister who stays with our aunt...youngest brother stays with me..."

(An eldest sister of 5 siblings, 25 yrs)

"...3 children were left when my daughter passed away...at first all of them stay with me...now I let middle child to stay with his aunt to help her with household chores...they stay in Mandalay..."

(A grandmother of 3 orphans, 55 yrs)

Education

Continuation of schooling

Majority (34 out of 41) were the orphans of school going age. About half of them could continue their schooling despite the economic hardship. Their continuation of schooling mostly depended on the economic condition of the family, child's health and support from some organizations and relatives. Some children join the non-formal education organized by WV.

"...I couldn't afford schooling of my grandsons but they want to attend the school...so I asked some acquaintance whether they can support for them. Now, both children can continue school. Some of our close relatives and friends give pens, pencils, books and WV arrange for school registration...."

(A grandfather of 2 orphans, 58 yrs)

"... My granddaughter can't study well. Her school performance is poor as she had to take medicines frequently. Now we let her to

study at non-formal education run by WV..."

(A grandmother of one HIV-positive orphan, 53 yrs)

Reasons for school drop out were different. Few HIV-positive children could not continue because of their health condition. Some dropped out because of their parents' health condition or economic hardship. Even though WV supported for school enrolment in formal schools, some of them dropped out after few months as their guardians could not afford other expenses for schooling.

"...My brother had to quit school before the final exam as we could not provide extra expenses for schooling like clothing, books, pens and some fees..."

(An elder sister of 13 years old orphan, 26 yrs)

Some elder children quit school because they had to work to earn money for younger siblings together with their single parents or extended family members. In one family, the elder child had to quit school as he had to look after his younger sister who was HIV positive.

"...The elder boy is 13 years old...he could not continue schooling as he had to take care of 6 years old younger sister who is HIV positive...he always accompany her to the clinic to take medicine..."

(A grandmother of 2 orphans, 66 yrs)

Stigma/Discrimination

Stigma/discrimination is not very common as most families rarely disclosed their family member's HIV status to their neighbors and relatives. Extended family members rarely discriminated the affected family since most of them stayed together for long time and they had same economic background. However, discrimination from neighbors was seen in some families. In addition, disclosure of the HIV status affected their working environment and opportunity. Discrimination was seen in spouse who was still alive and infected.

Few volunteers also stated the problem of discrimination. However, many community volunteers stated that the affected families rarely disclosed the HIV status to their neighbors.

“...I have two sons...the elder boy has some problems with neighbors...he frequently fights with other children as they make a joke with his mother’s death...they said...she died of “A” (အေ ဂိုဏ်းဝယ်)... my son can not tolerate and fights back... some children threw with stones to our house...”

(A father of 2 sons, 39 yrs, HIV positive)

“...I work as a street vendor...sometimes I sell snack...sometimes fruits...I can’t sell in my ward...as they know my disease...but I can sell in other wards as they don’t know...”

(A mother and an aunt of 3 orphans, 25 yrs, HIV positive)

Economic consequences

Economic consequences were mainly focused on change in family possession and effect on daily living. Majority was from low socio-economic background before they got HIV infection and thus change in family possession was not so significant after getting the disease. However, along with the loss of the bread winner in some families, they found difficult to struggle for family’s daily expenses. Consequently, spouse and older children needed to work to earn money by doing odd jobs (*kya-ban*). Many families needed to sell household assets including cooking utensils, clothings, etc. In extreme case, spouse worked as a commercial sex worker for her family’s living.

“...Before my husband died, I don’t need to work. Now, I work as manual labour in day time and I also work as sex worker at night...about 2-3 times per week...”

(A mother and an aunt of 3 orphans, 25 yrs, HIV positive)

“...We’ve faced difficulty for living after my husband’s death as woman’s income can’t compare with that of man. Sometimes if

I can’t work...we’ve to pawn the cloths in exchange with money...”

(A mother of 4 orphans, 37 yrs, HIV positive)

Health consequences

Of all health consequences, transmission of HIV from mother to child was the most severe one. Six children got HIV from their mother and two children already died before their 5th birthday. Common health problems among HIV infected children were malnutrition and TB. Consequently, they took the treatment frequently and sometimes required hospitalization. Some HIV-positive orphans and single parents are taking anti-retroviral therapy (ART) with the support of AZG. Some guardians came to know the children’s HIV status only when they had to seek health care for TB. Other HIV-negative orphans were healthy apart from some minor illnesses like skin infection and worm infestation. They normally took treatment from “Thazin” clinic run by AZG.

“...She suffers from malnutrition frequently. She is very thin and...has to take treatment at Thazin...she stays there at day time... they provide her with nutritious food...”

(A grandmother, 53 yrs)

Support networks

Support from extended families, relatives and neighbors

The most important support from the extended families especially grandparents was to share a place to live. Immediate relatives supported for their daily living since many of them stayed together in the same house or compound. Supports from other relatives and neighbors were not regular and inadequate for their expenses. In some families, grandparents had to work outside in order to support the orphans.

“...We stay with my sister’s family...we can eat together but they can’t support us sometimes other relatives give me some money like 2-3000 kyats ...”

(A mother of 2 orphans, 25 yrs)

“...We stay in grandmother’s house together with my mother and aunts...at first in a same house...now we stay in a small hut in-front but in the same compound. My mother works outside and supports us....”

(A mother of 2 orphans, 32 yrs, HIV positive)

Support from organizations

Different organizations have their own agenda to support the affected families. Organizational support can be categorized as education, health care and financial support. Some BHS participated in these activities although it was not done in collaboration with public sector. Many public health care providers stated that these programs were beneficial for the affected families although sustainability was uncertain. In contrast, some of them concerned for the problem of increasing migrant population in their township because of these programs.

“...There’s a nutritional program for the children organized by WV, I’ve to recruit and weigh the children for their program. They invite me to participate as responsible health personnel. They provide lunch to the children...I also try to contribute by giving medicines for minor ailments...”

(A midwife, 48 yrs)

Education support

In the study township, WV supports school enrollment of the children. They arranged the program not only for the HIV/AIDS orphans but also for the children from the poor families. Additionally, these orphans can join the non-formal education program. Vocational training for HIV-positive children is also provided from WV and AFXB, an INGO working for PLWHA.

“...It’s for the children who can’t attend the formal school...We accept 7-15 years old children... the class usually starts from 9:00 am to 3:00 pm. We teach them till they can read and write...mainly for Myanmar, English and Mathematics....”

(A volunteer, 25 yrs, 5 yrs service)

“...We have a link with other INGO like AFXB. We send some HIV orphans and children from poor families to attend the vocational training in which girls can learn for sewing, knitting and boys can learn for carpentry...”

(A volunteer, 57 yrs, 10 yrs service)

Health care

Majority of the affected families in the study area usually seek health care at Thazin clinic run by AZG. Both HIV infected orphans and their infected parents took treatment in these clinics. The clinics provide antiTB drugs for all TB patients from the affected families and ART free of charge for some infected individuals.

Regarding general health, children can take treatment from the clinics that have been linked with WV. If they need to take treatment at the hospital, WV arranges and provides the cost.

“...My grandson contracted TB and took treatment at Thazin...now it’s finished... now he needs to take only medicines for “A”...”

(A grandfather of 2 orphans, 58 yrs)

“...If a child needs to seek health care...I’ve to inform my superior and ...there’s a token from WV...by using the token...he/she can take treatment from those clinics...”

(A volunteer, 29 yrs)

Financial support

WV supports financially by giving loans for income-generation. There are some rules to control misuse of the opportunity. Receivers have to recruit a group of five people to guarantee among themselves. If someone can not give the money back on time, other group members have to pay for that person. Some are reluctant to apply for that kind of loan since they do not want to guarantee for others although they want to borrow money for themselves. Moreover, the program is not so beneficial for those who are very poor as others do not want them to join the group.

“...To apply loan from WV...we must have a group to guarantee each other. Others do

not want to accept like us in their group since we are poor and they think that we can't give back on time..."

(A mother of 5 orphans, 43 yrs, HIV positive)

DISCUSSION

The study was done in Hlinethaya Township having community-based activities focusing on HIV/AIDS for about 5 years. Using qualitative research methods, socioeconomic and health consequences faced by HIV/AIDS orphans were identified. Moreover, support networks for the affected families were described.

Family dispersion, effects on education, stigma/discrimination and psychological consequences were observed as the common social consequences. Of all social consequences, drop out from school was the most significant finding among the affected families. Only half of the school going age children could continue the formal education. This finding of effect on education supported the previous study but the magnitude of drop out from school was much higher than that of previous one [9]. Findings from the African studies identified that orphans are at risk of poorer educational outcomes with maternal deaths having stronger negative effects than paternal deaths [10].

Staying with extended family members was common finding not only for double orphans but also for paternal/maternal orphans. It may contribute from the Myanmar tradition of staying as extended families. Discrimination from neighbors was seen in some families but rarely observed in school environment. This may be due to the concealment of the HIV status of the family members in the schools attending by orphans.

Regarding the economic consequences, change in family possession was not so significant in most of the families. Underlying poverty of the affected families in the study township may partly contribute to that finding. In contrast, significant change in

family possession was seen in the previous study [9]. Only prominent change was difficulty in struggling for family's daily expenses along with the loss of the bread winner in some families.

Transmission of HIV from mother to child was identified as the most serious health consequence faced by the children. Lack of awareness of HIV positive status and delayed seeking care of infected mother may contribute to that occurrence of transmission.

Majority of affected families received benefits from the support programs of INGOs especially for education and health care. Non-formal education programs and vocational trainings seemed to be beneficial for those who can not afford the school expenses. However, loans for income generation could not help much for poor families.

Although many providers from public sector appreciated the benefits of support programs for the affected families, some of them pointed out about the sustainability. Moreover, few concerned of increasing migrant population because of support programs in their township.

Our study highlights the situation of HIV/AIDS orphans and their families in the community. All affected families have some extent of socio-economic consequences from HIV/AIDS. Since availability of support networks from INGOs could alleviate the negative consequences faced by the affected families, sustainability and strengthening of these programs should be strongly considered.

Recommendations

Further study with larger sample size using both quantitative and qualitative research methods should be conducted in order to identify and compare the situation of HIV/AIDS orphans in different localities.

Non-formal education and vocational training programs should be strengthened and expanded. It was beneficial for the

orphans who can not afford the formal education. Moreover, majority of the guardians want their children to get vocational skills to earn money for their living in the future. Community and public sector involvement should also be promoted for sustainability of these education programs.

Furthermore, coordination between community, public sector and INGOs should be encouraged for implementation of better rehabilitation programs for HIV/AIDS orphans in the future.

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**Analysis of haemoglobinopathies and haematological parameters
in healthy children living in delta region of Myanmar**

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The aim of this study is to analyze the abnormal haemoglobin types and haematological parameters in total 100 apparently healthy children aged 5-12 years of both sexes who are living in delta region of Myanmar. The results showed that HbA (normal Hb) was detected in 65 children (65%) including 32 males and 33 females, HbE (homozygous) was seen in 5 male subjects (5%) and HbEA (heterozygous) was found in 30 children (30%) including 16 males and 14 females by isoelectric focusing (IEF). Normal reference haematological parameters from automatic CP coulter (Pentra-60) are 11-18 g/dl for Hb concentration, $4-6.2 \times 10^6/\text{mm}^3$ for red blood cell count, $80-100 \mu\text{m}^3$ for mean MCV and 26-34 pg for mean MCH. The mean Hb concentration was 11.93 g/dl in HbA, 11.12 g/dl in HbEA and 10.56 g/dl in HbE in this study. The mean MCV was $79.35 \mu\text{m}^3$ in HbA, $73.3 \mu\text{m}^3$ in HbEA and $60.8 \mu\text{m}^3$ in HbE. The mean MCH was 25.72 pg in HbA, 22.6 pg in HbEA and 18.82 pg in HbE. The mean red blood cell count was $4.44 \times 10^6/\text{mm}^3$ in HbA, $4.58 \times 10^6/\text{mm}^3$ in HbEA and $4.98 \times 10^6/\text{mm}^3$ in HbE. Morphology of red cells in blood film showed normal features in HbEA and hypochromic microcytic and poikilocytosis with target cells in HbE. Mean haematological parameters including Hb, MCV and MCH of HbEE were lower than these parameters of normal HbA except total red cell counts. According to the final analysis of haemoglobinopathies, HbEA (heterozygous) was six times higher than HbEE (homozygous) type among the healthy children living in delta region of Myanmar.

INTRODUCTION

Haemoglobinopathies (haemoglobin disorders) are conditions of abnormal haemoglobins and anaemia that are produced by defected genes to control the haemoglobin (Hb) synthesis. Abnormal haemoglobins are HbS, HbC, HbE, HbH and Hb Barts [1]. HbE is one of the commonest haemoglobinopathies in the world that is a heterogeneous group of disorders whose phenotypes range from asymptomatic to severe. HbE is a variant haemoglobins with a mutation in the β globin gene caused by substitution of glutamic acid for lysine at position 26 of the β globin chain [2].

HbE trait (EA) and HbE (EE) are mild disorders and E beta thalassaemia is the most serious form of E syndromes, produced in a million of people worldwide especially in North America. The World Health Organization (WHO) estimates that 100,000 new cases of HbE β thalassaemia are expected in Thailand for the next few decades. High prevalence of HbE is predicted for India, Sri Lanka, Malaysia and Southern China [3].

HbE is common in South-East Asia where its prevalence can reach 30-40% in Thailand, Cambodia and in Laos. One study in India showed that HbE carrier was 45.85%, homozygous HbE was 14.83%,

E beta was 3.87% and E beta thalassaemia was 1.93% among population of this area [4]. In Myanmar, the prevalence of HbE beta thalassaemia varies with indigenous race or ethnicity according to the previous research findings in which it was higher prevalence in Kayin indigenous race than others (8.9% vs. 2.3%). The prevalence of HbE is higher in Chin indigenous race than others (53% vs. 20%) [5].

HbE variants can be detected by agarose gel electrophoresis, isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) [6]. The blood films of HbEE show microcytic with target cells that may be confused with thalassaemia or initial stage of iron deficiency anaemia. Homozygous HbE and E beta thalassaemia have severe anaemic picture with morphological changes of red cells and many target cells. Heterozygote HbE has absence of anaemic picture but it has elevated red cell count and presence of target cells in blood film [5]. Therefore, this study was performed to identify the types of haemoglobin and to determine haematological parameters in healthy children living in Bogalay Township, delta region of Myanmar.

General objective

- To analyze the haemoglobin types and haematological parameters in apparently healthy children living in delta region of Myanmar.

Specific objectives

- To determine the haematological parameters (complete blood picture) of healthy children by using the fully automated haematological analyzer (Pentra-60)
- To observe the morphological pattern of red blood cells in blood film examination for anaemia-positive children
- To find out the different types of haemoglobin by isoelectric focusing (IEF) electrophoresis
- To analyze the abnormal haemoglobin types and haematological parameters of healthy children living in delta region

MATERIALS AND METHODS

A cross-sectional, community-based descriptive study was conducted in apparently healthy children living in Bogalay Township, the delta region of Myanmar.

A total of one hundred healthy school children within the age of 5-12 years and both genders were recruited and tested for haematological parameters and different haemoglobin types in one year period. The children with other haematological and other diseases were excluded in this study. After approving by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar), informed consents were obtained from parents or guardians of these children living in two villages of Bogalay Township.

Three milliliters (ml) of whole blood from antecubital vein of all subjects were collected into ethylene diamine tetraacetate (EDTA) contained K3 test tube, mixed thoroughly, and kept at ice packed within 24 hours until testing in the Pathology Research Division.

All samples were tested for complete blood cell count (CBC) by fully automated hematological analyzer ABX Pentra-60. The whole blood 10 µl was mixed with 10 µl of 1% saponin solution for at least 3 hours at room temperature before running the isoelectric focusing electrophoresis at pH 6.0-6.2 that can identify the different types of haemoglobin in the blood by separation of globin chain. All whole blood samples were stained with Leishman and the blood film examination in morphology of red cells, white cells, platelets and other atypical blood cells was done by using the high power of light microscope.

The results obtained throughout the study were recorded according to the proforma and all data were calculated for odds ratios (Ors), p-value, confidence interval (CI) by using the equality of populations (Kruskal-Wallis rank sum test) in this study.

RESULTS

Clinical and haematological characteristics of subjects

Total number of healthy children was 100, of which 53% were males and 47% were females living in villages of Bogalay Township. In this study, the mean age of female healthy children was slightly higher than that of male children (8.64 ± 2.01 vs. 8.34 ± 1.98 years). The commonest age group was found in 7-8 years (29 out of 100) children.

Table 1. Distribution of different haemoglobin types by age group in healthy children

Age range (yrs)	Different haemoglobin types of healthy children			Total number of children
	Hb 'A'	Hb 'EA'	Hb 'EE'	
5-6	12	10	2	24
7-8	17	10	2	29
9-10	19	5	1	25
11-12	17	5	-	22
Total	65 (65%)	30 (30%)	5 (5%)	100

Mean age of healthy children = 8.34 ± 1.98 yrs in males
= 8.64 ± 2.01 yrs in females

The different haemoglobin (Hb) types were found in 65 (65%) in haemoglobin A, 35 (35%) in haemoglobin EA and 5 (5%) in haemoglobin EE, respectively. The highest incidence of HbA (normal Hb) was found in 9-10 years age group (19/65) and the commonest age groups of HbEA and HbEE were observed in 5-6 years and 7-8 years age groups (10/30 vs. 10/30 and 2/5 vs. 2/5, respectively) in this study (Table 1).

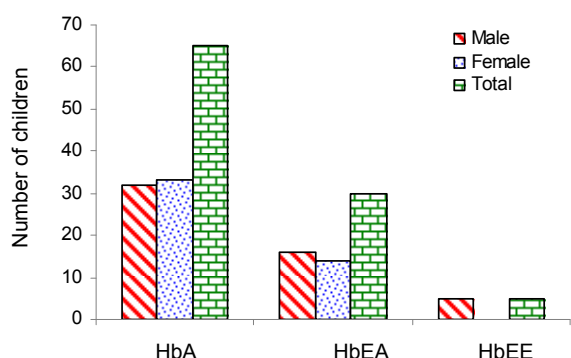


Fig. 1. Results of sex distribution and different haemoglobin types in healthy children

Haemoglobin 'A' (normal haemoglobin) was detected in 32 males and 33 females, HbEA was found in 16 males and 14 females and HbEE was identified only in 5 males of healthy children by using the isoelectric focusing gel electrophoresis (Fig. 1).

Table 2 shows the mean haematological parameters related to red blood cells in children with presence of anaemia and absence of anaemia. The results of mean haemoglobin value, mean corpuscular volume, mean corpuscular hemoglobin and mean haematocrit of anaemic children were lower than those of non-anaemic children in both sexes. The mean total red blood cell count, white blood cell count and mean corpuscular haemoglobin concentration were not significantly different in both sexes. The result of mean red cell distribution width was more increased in anemic children than in the non-anemic children of both sexes in this study.

Table 2. Mean haematological parameters of anaemic and non-anaemic children by automated haematological analyzer (Pentra-60)

Haematological parameters	Male children		Female children	
	Anaemia (+)	Anaemia (-)	Anaemia (+)	Anaemia (-)
Haemoglobin (Hb) (11-18) g/dl	10.27	11.83	9.32	11.77
Total red cell count (RBC) ($4-6.2 \times 10^6/\text{mm}^3$)	4.52	4.54	4.53	4.51
Mean corpuscular volume (MCV) ($80-100 \mu\text{m}^3$)	73.72	79	75.10	80.29
Mean corpuscular haemoglobin (MCH) (26-34) pg	24.66	25.64	23.85	26.09
Mean corpuscular haemoglobin concentration (MCHC) (31-35) g/dl	31.42	31.94	30.21	32.50
Haematocrit (HCT) (35-55)%	31.39	35.96	32.58	36.18
Red cell distribution width (RDW) (10-20)%	14.91	12.52	14.32	13.54
Total white blood cell count (TWBC) ($4-11 \times 10^3/\text{mm}^3$)	6.90	6.23x10	7.75	6.74
Total platelet count ($150-400 \times 10^3/\text{mm}^3$)	271.09	237.48	260.32	226.26

Table 3 shows different grades of anaemia based on levels of haemoglobin in children according to WHO classification [7, 8]. In

the present study, severe anaemia was not detected but moderate anaemia was found in 10 males (66.67%) and 5 females (33.33%) and mild anaemia was determined in 12 males (44.44%) and 15 females (55.55%), respectively.

Table 3. Different grades of anaemia in children based on WHO classification

Haemoglobin concentration (11-18 g/dl)	Different grades of anaemia	No. of males (%)	No. of females (%)	No. of children (%)
<7 g/dl	Severe anaemia	-	-	-
7.1-10 g/dl	Moderate anaemia	10 (66.67)	5 (33.33)	15
10.1-12 g/dl	Mild anaemia	12 (44.44)	15 (55.55)	27
Total		22(52.38)	20(47.61)	42

Prevalence of anaemia

Anaemia is defined as reduction in concentration of haemoglobin level in the peripheral blood below the lower limit of normal range for the age and sex. The haematological parameters including haemoglobin concentration were determined by automated blood analyzer (Pentra-60). The normal reference value is 11-14 g/dl for children under 12 years of age in both sexes. In this study, anaemia was found in 42 (22 male and 20 female) children with absence of clinical sign and symptom. Otherwise, 58 children (31 males and 27 females) living in villages of Bogalay Township had absence of anaemia.

Detection of haemoglobin types

Total 100 apparently healthy children were detected the different types of haemoglobin by isoelectric focusing electrophoresis. In this study, normal haemoglobin 'A' band was detected in 23 males (54.76%) and 14 females (33.33%) of anaemic children. Haemoglobin E and A bands were identified in 7 males (31.8%) and 7 females of anaemic children. Haemoglobin E band was only detected in 5 male anaemic children. (Fig. 2). Twenty-three out of 42 (54.76%) non-anaemic children showed normal Hb (HbA) type and 14 subjects (33.33%) had

HbEA. HbEE was not detected in non-anaemic children in this study.

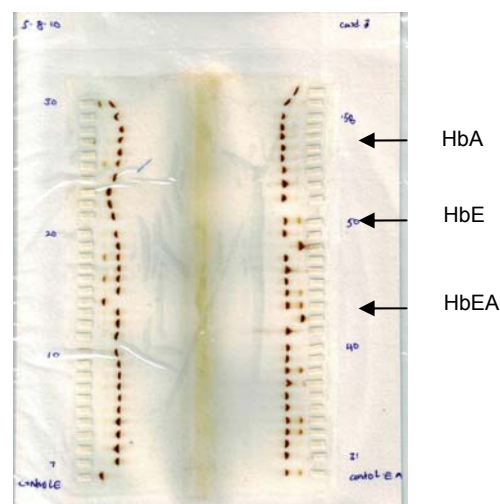


Fig. 2. Detection of haemoglobin bands in whole blood samples of children by isoelectric focusing electrophoresis method.

Analysis of haematological parameters related to the different types of haemoglobin

The mean value of haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of HbE were less decreased than those of HbA and HbEA types. The total red blood cell count and red cell distribution width of HbE were the highest values in this study (Table 4).

Table 4. Results of haematological parameters related to the different types of haemoglobin in apparently healthy children

Mean values of haematological parameters related to red blood cell	Haemoglobin 'A'	Haemoglobin 'EA'	Haemoglobin 'EE'
Haemoglobin (11-14) g/dl	11.93±1.59	11.12±1.056	10.56±1.045
Total red blood cell count (4-6.2)x10 ⁶ /mm ³	4.44±0.39	4.58±0.44	4.98±0.42
Mean corpuscular volume (80-100) µm ³	79.35±6.29	73.3±4.11	60.8±1.304
Mean corpuscular haemoglobin (26-34) pg	25.72±2.39	22.6±3.22	18.82±0.683
Mean corpuscular haemoglobin concentration (31-35) g/dl	30.34±1.79	31.9±0.644	30.9±0.505
Red cell distribution width (10-20)%	13.74±1.658	14.65±1.812	15.38±1.599

The haematological parameters of 100 apparently healthy children were analyzed by equality of populations (Kruskal-Wallis rank sum test). All haematological parameters related to red blood cells of children had significant value ($p < 0.05$) in this study. The probability results of haematological parameters (p value) were 0.0086 in haemoglobin concentration, 0.0073 in total red blood cell count, 0.0045 in red blood cell width, respectively. p value was 0.0001 in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.

DISCUSSION

In this study, haemoglobin concentration was lowest in HbEE type of apparently healthy children and the highest concentration of haemoglobin in HbA (normal Hb type). Anaemia was found in 42% of children of both sexes who were determined by automatic haematological analyzer. Fifty-eight children had absence of anemia. Haematological parameter of anaemia was not significantly different in male and female subjects but these parameters were significantly different between presence and absence of anaemia in healthy children.

Measurement of Haemoglobin concentration (Haematocrit) is the most cost efficient and commonly used method for screening of anaemia. The most common causes of anaemia in children are iron deficiency anaemia or nutritional anaemia. The other causes of anaemia are sickle cell trait, hemolytic anemia like thalassaemia minor and major, parasitic infections, folate and vitamin B₁₂ deficiency and chronic inflammatory diseases [9].

The lowest value of mean corpuscular volume (MCV) was found in HbEE type among all subjects in this study. Other study reported that the MCV was significantly lower in all age groups and hypochromic microcytic anaemia was identified in 13.6% of healthy children and the mean Hb level

was 13.5 ± 1.3 g/dl in males and 13.4 ± 1.28 g/dl in females under 12 years of age [10]. The mean red cell count was more or less constant in the age of 1-13 years old with both sexes in this research study [11]. The prevalence of hypochromic microcytic anaemia was increased in apparently healthy children who were living in post-cyclone area at Bogalay Township, delta region of Myanmar. Total red blood cell count and white blood cell count were not significantly different between presence and absence of anaemia. The red cell indices did not show any significance between the male and female subjects. Some anaemic children had mild thrombocytosis in both results of automated blood analyzer and blood film examination with Leishman stain observed by high power of light microscope.

In previous research study of Myanmar, the prevalence of HbE (EE) was 11.4% in Kayin-predominant villages and 13.1% to 24.4% in the Bamar-predominant villages. Therefore, the prevalence of HbE was strongly associated with influence of racial differences in Myanmar [5]. The structural haemoglobin variants are typically based on point mutation in a globin gene that produce a single amino acid substitution in a globin chain that produce homozygous HbC, HbH (alpha thalassaemia) and HbS (sickle cell disease) and homozygous HbE (beta thalassaemia) and HbD disease. The structural haemoglobinopathies depend on the red cell indices and complete blood cell count (CBC) including Hb, red blood cell (RBC) number, mean corpuscular volume (MCV) and red cell distribution width (RDW). All haematological automatic analyzers can measure these parameters precisely and accurately by using computer software system. Most reference values of haematologic indices are varied depending on many factors such as genetic factors, ethnic origin, gender and environment factors especially for altitude and season. Among them, genetic factor is the most increased variance (61%-96%) [12]. IEF is a electrophoretic technique with excellent resolution that has been used to identify the Hb variants such as

HbC, HbE, HbA, HbF, HbS, HbD, HbG according to resolution and migration of haemoglobin in a pH gradient. Although it is labor-intensive and time-consuming, it identifies the accurate narrow bands at gel electrophoresis [13].

A previous study showed a global prevalence of 16.6% of abnormal haemoglobin (haemoglobinopathies) carriers of blood donors in the Mauritanian population. In the black population, the prevalence of haemoglobinopathies is higher than white population (21.44% vs. 4.47%) in this area [14]. HbE trait was detected in 20% of blood donors from National Blood Center in Myanmar [15]. A study in India showed that the overall prevalence of haemoglobinopathies was 13.1% in population among the two districts, HbE trait (HbAE) was found 2.7% and HbE disease (HbEE) was 0.3% in their population [7]. In Myanmar, HbE carrier prevalence rate was 19.09% (18.09% in HbEA and 1% in HbEE) in healthy school children aged 5-12 years in Yangon [16].

This study may provide the reference red blood cell indices in apparently healthy children and correlation of haemoglobinopathies and haematological parameters of anaemic children. Therefore, early detection of haemoglobinopathies is very important program in National Health Plan for premarital, antenatal and neonatal health units in Myanmar.

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**Acute and sub-acute toxicity of *Cuminum cyminum* Linn.
(Ziya) seeds on laboratory rats**

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Cuminum cyminum Linn. (Ziya) seed is one of the most useful spices in Myanmar. It is included in curry powder and some traditional medicine formulations. In acute toxicity test, it was found that there was no toxic symptom in albino mice at the dose of up to 4 gm/kg body weight. In sub-acute toxicity test, three groups of rats were tested orally once daily for 90 days. Group 1 and Group 2 received 3 gm/kg and 1 gm/kg body weight of seed powder. Group 3 received only 10 ml/kg of distilled water and served as control group. On day 91, all three groups were sacrificed and blood was collected for biochemical tests (LFT and urea) and haematological parameters. Internal organs were dissected out, weighed and histopathological examinations were done. Sub-acute toxicity test showed that there were no changes of body weight and organ weight in all three groups. There were no significant changes in LFT, urea, complete picture and platelet count when compared with the control group. In histopathological examinations, squamous metaplasia, necrosis and polymorph infiltration were observed at mucosa of small intestine in some high dose treated rats (3 gm/kg body weight). Mild haemorrhage in kidney was also seen in 3 out of 8 rats. There were no significant changes of histopathological examinations in low dose and control groups.

INTRODUCTION

Cuminum cyminum Linn. (Ziya) is a small, slender annual herb, which is grown extensively in South-Eastern Europe, North Africa bordering the Mediterranean-sea, in India and China. It is cultivated in almost all the states in India. The plant prefers a mild climate and grows from sea level up to an elevation of 10,000 feet. Seeds have an aromatic odour and a spice somewhat bitter in taste, and are extensively used as condiment. This plant is commonly known as cumin and locally it is called Ziya. In Indian medicine, cumin seeds have long been considered as stimulant and carminative. They are stomachic, astringent and useful in diarrhoea and dyspepsia. They are now chiefly used in veterinary medicine [1]. *C. cyminum* is one of the popular spices

regularly used as a flavouring agent in a number of ethnic cuisines. In Iranian ancient medicine, the fruits have been used for treatment of toothache, diarrhoea and epilepsy. *C. cyminum* is also emerging as an alternative antimicrobial agent that is safe for human applications [2]. Some researchers revealed that *C. cyminum* seeds have hypolipidemic effect, hypoglycemic effect, anti-nociceptive and anti-inflammatory effects [3, 4 & 5].

There was a report on only acute toxic effect of *Cuminum cyminum* Linn. (Ziya) seeds [6]. Sub-acute toxicity of *Cuminum cyminum* Linn. (Ziya) seed powder has never been reported in available literature. Therefore, this study was undertaken to investigate the acute and sub-acute toxicity of *C. cyminum* Linn. seeds on mice and rat models to gain some toxicological information which is

necessary for further experiment or future application in human trial.

General objective

- To investigate the acute and sub-acute toxicity of *Cuminum cyminum* Linn. (Ziya) seeds on animal model.

Specific objectives

- To find out the phytochemical constituents of dried seeds of *Cuminum cyminum* Linn.
- To determine the acute toxicity of dried seed powder on mice
- To test the sub-acute toxicity of dried seed powder on rats

MATERIALS AND METHODS

Plant materials

Dried seeds of *Cuminum cyminum* Linn. were purchased from a herbal shop in Yangon. They were ground into fine powder by grinding machine and then the powder was stored in a glass bottle with cap at room temperature.

Reagents and chemicals

All the reagents and chemicals used were of analytical grade.

Phytochemical screening

Screening for phytochemical constituents such as alkaloids, flavonoids, glucosides, etc. was done according to the method of Harborne JB, and Hakin MA [7, 8].

Animals

Laboratory mice and rats were obtained from the Laboratory Animal Services Division, Department of Medical Research (Lower Myanmar). Fifty healthy albino mice (ddy strain) of both sexes with an average weight of 30-35 gm were used for acute toxicity test. Twenty-four healthy albino rats (Wistar strain) of both sexes with an average weight of 180-200 gm were acclimatized for about 2 weeks in the laboratory. The animals had free access to standard pellet diet (DMR/LM) and water *ad libitum*.

Study site

Pharmacology Research Division and Laboratory Animal Services Division, Department of Medical Research (Lower Myanmar), Yangon.

Study period

One year (2009-2010)

Study design

Control parallel study design was used.

Acute toxicity test

Acute toxicity test was done to determine the lethal dose 50 (LD₅₀) according to the method of Litchfield and Wilcoxon [9]. Fifty albino mice in 5 groups (10 mice/group) were given orally the solution of dried seed powder of *Cuminum cyminum* Linn. (Ziya) in distilled water at the dose of 1, 2, 3 and 4 gm/kg body weight. The control group received 10 ml/kg body weight. All the animals were kept under observation for toxic symptoms for two weeks.

Sub-acute toxicity test

Sub-acute toxicity test was done according to the method of Sethi N [10]. The rats were randomly divided into three groups of 8 rats each. Group 1 and Group 2 received 3 gm/kg and 1 gm/kg body weight of dried seed powder dissolved in distilled water once daily orally for 90 consecutive days. Group 3 received 10 ml/kg of distilled water only and served as control group.

During the period of 90 days, body weight was weekly recorded and all the rats were closely observed for signs of physical abnormalities. On day 91, all the rats were anaesthetized with chloroform and blood samples were immediately collected by cardiac puncture for biochemical tests (LFT & urea) and haematological parameters (complete picture and platelet count). Necropsy was performed to examine gross pathological lesions of internal organs. Internal organs such as brain, heart, lungs, liver, stomach, kidneys, spleen, pancreas and small intestine were excised, weighed

and then preserved in 10% formalin solution for histopathological examinations.

Statistical analysis

Data were analysed statistically using Students 't' test. The results were expressed as the mean $\pm$ SEM. The significance of the differences between control and test groups was determined by the Students 't' test and values of $p < 0.05$ were taken to imply statistical significance.

RESULTS AND DISCUSSION

The phytochemical tests revealed that dried seed powder of *Cuminum cyminum* Linn. included alkaloids, flavonoids, glycosides, tannins, steroids, phenols, saponins, amino acid and resin. Triterpine and cyanogenic glycoside were not included (Table 1).

Table 1. Results of phytochemical tests of *Cuminum cyminum* Linn. (Ziya) dried seed powder

No.	Compound	Test reagent used	Observation	Results
1	Alkaloids	Dragendorff 's Reagent	Yellow ppt	+
2	Flavonoids	Mg turning, conc: HCl	Pink colour	+
3	Glycosides	10% NaOH, 1ml distilled water	Yellow colour	+
4	Tannins	10% FeCl ₃ + dil H ₂ SO ₄	Yellowish-brown ppt	+
5	Steroids	Acetic anhydride, conc. H ₂ SO ₄	Green to blue colouration	+
6	Phenols	10% FeCl ₃ , distilled water	Green colour	+
7	Saponins	EtOH+conc. H ₂ SO ₄	Red colour	+
8	Amino acid	2% Ninhydrin reagent	Dark violet colour	+
9	Resin	Acetic anhydride, conc. H ₂ SO ₄	Violet colour	+
10	Triterpine	Acetic anhydride, conc. H ₂ SO ₄	No pick colour	-
11	Cyanogenic glycoside	Sodium picrate paper	No brown colour	-

ppt = Precipitate += Present -= Absent

In another study, aqueous and 80% ethanol extracts of *C. cyminum* seeds also showed the same phytochemical constituents except resin. LD₅₀ of aqueous and 80% ethanolic extracts of Ziya seeds was reported comparable to more than 16 gm/kg body weight [6]. This dose is equivalent to 58 gm/kg body weight of dried seed

powder. In this study, there were no death or any sign of toxicity observed after oral administration of single doses of dried seed powder up to the maximal permissible dose of 4 gm/kg body weight. Some adverse effects, such as hyperactivity, asthenia, anorexia, diarrhoea, sedation and syncope were not seen after the highest dose of 4 gm/kg body weight. Therefore, the LD₅₀ of *C. cyminum* dried seed powder determined in mice of both sexes after acute oral administration was supposed to be more than 4 gm/kg body weight.

In the sub-acute toxicity test, dried seed powder treated groups did not produce any lethality when the doses of 1 gm/kg and 3 gm/kg body weight were administered orally daily for 90 days. The body weight and organ weight of the treated groups were not statistically different from those of the control group. There were no significant changes in SGOT, SGPT, alkaline phosphatase, bilirubin and urea, when compared with the control group (Table 2).

Table 2. Results of biochemical tests in control and *Cuminum cyminum* Linn. (Ziya) dried seed powder treated rat groups

Group	Bilirubin (μ mol/L)			Alkaline phosphatase (μ mol/L)	SGOT IU/L	SGPT IU/L	UREA mg (%)
	Total	Conjugated	Unconjugated				
Control (D/W)	9.12 $\pm$ 0.12	6.0 $\pm$ 0.1	3.12 $\pm$ 0.1	137.5 $\pm$ 1.5	220.25 $\pm$ 1.5	63.5 $\pm$ 1.5	38.37 $\pm$ 1.5
10ml/kg b.w	1.13 $\pm$ 0.03	0.93 $\pm$ 0.03	0.40 $\pm$ 0.03	13.75 $\pm$ 0.75	19.88 $\pm$ 0.75	8.78 $\pm$ 0.75	1.04 $\pm$ 0.75
1gm/kg b.w	10.62 $\pm$ 0.68	7 $\pm$ 0.57	3.62 $\pm$ 0.32	132.12 $\pm$ 18.44	166.37 $\pm$ 26.90	63.5 $\pm$ 3.25	39.87 $\pm$ 2.71
3gm/kg b.w	12.12 $\pm$ 1.32	7.75 $\pm$ 0.36	4.37 $\pm$ 0.63	159.37 $\pm$ 19.08	157.5 $\pm$ 25.02	56.0 $\pm$ 6.33	35.37 $\pm$ 1.49

Values are expressed as mean $\pm$ SEM.

n=8 in each group

* $p < 0.05$ when compared to control

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) showed normal values within the limit of variation. However, decrease in SGOT was observed at the high dose (3 gm/kg body weight) but not significant. Serum alkaline phosphatase, bilirubin and urea also showed no significant change in control group and tested groups.

In addition, there were no significant changes in haematological parameters such as complete picture and platelet count between the treated groups and the control group (Table 3). No enlargement and no remarkable gross lesion were found in internal organs of all three groups.

Table 3. Haematological parameters of control and *Cuminum cyminum* Linn. (Ziya) dried seed powder treated rat groups

Haematological parameters	Control group	1 gm/kg body weight treated group	3 gm/kg body weight treated group
Hb (g/dl)	13.71±0.98	15.03±0.49	14.33±0.18
WBC (×10 ³ /μl)	6537.5 ±0.986	10012.5±1481.84	7687.5±1066.4
Lymphocyte (%)	72.12±2.79	76.5±1.53	76.5 ±2.36
Neutrophil (%)	20.75±2.05	17.25 ±1.17	18.75 ±2.06
Monocyte (%)	3.0±0.47	3.0±0.38	4.75 ±0.73
Eosinophil (%)	1.42± 0.26	1.0±0	1.25 ±0.17
Platelet count (×10 ³ / μl)	656.75±112.03	901.5±17.79	682.5 ±129.20

Values are expressed as mean±SEM.

n=8 in each group.

*p<0.05 when compared to control.

In histopathological examinations, no histopathological abnormalities of selected organs were seen in both control group and low dose treated group (1 gm/kg b.wt). In some high dose treated rats (3 gm/kg b.wt), squamous metaplasia, necrosis and polymorph infiltration at mucosa of small intestine were observed (Fig. 1).

Although histopathological changes were observed in small intestine, no sign of gastrointestinal changes such as vomiting, bloody stool and loose motion were observed. Mild haemorrhage in kidney was also seen in some rats (3 out of 8 rats) but there were no significant changes in urea when compared to the control group. This may be due to the individual or combined action of phytoconstituents present in the dried seed powder or may be the spicy effect of dried seed powder of *C. cyminum*. The component responsible for this effect, however, was not investigated. These

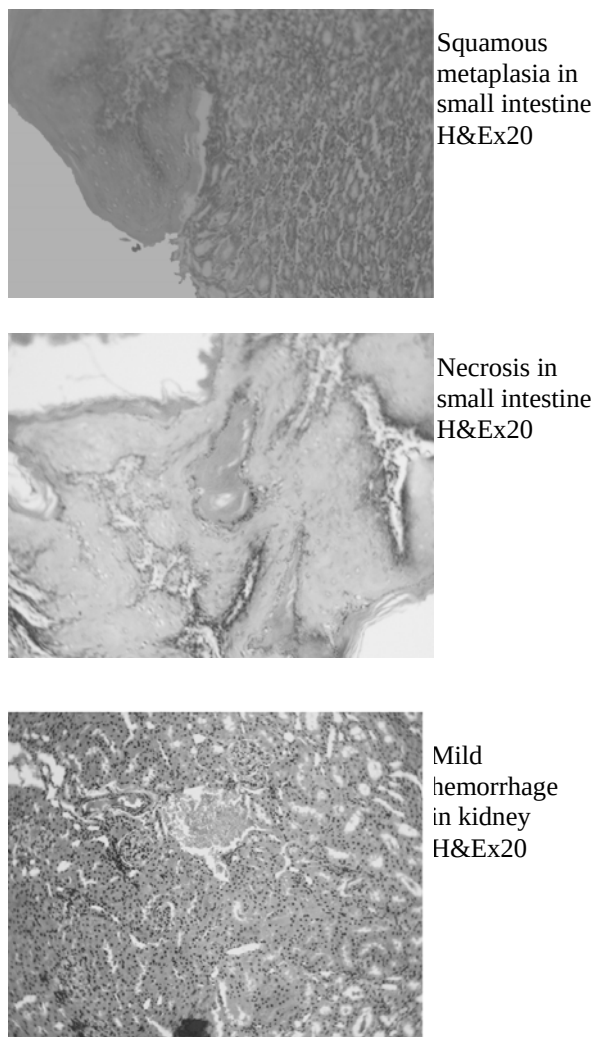


Fig. 1. Histological sections of small intestine showing squamous metaplasia and necrosis and section of kidney showing mild haemorrhage

investigations were intended to reveal possible toxic effects of *C. cyminum* Linn. It was shown that toxic effect of *Cuminum cyminum* Linn. occurred at higher dose than those employed in the traditional medicine formulations. In conclusion, these findings showed that dried seed powder of *Cuminum cyminum* Linn. (Ziya) has sub-acute toxicity when administered orally for 90 days consecutively to laboratory rats at the dose of 3 gm/kg body weight.

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**Toxicity study, isolation and identification of gelsedine
from toxic plant, *Gelsemium elegans* Benth.**

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Since plants contain multiple chemicals and classes that work independently or in concert, understanding plant toxicity is often poor. Additionally, plant themselves are inherently variable and potency and type of toxin depend on the season, geography, local environment, plant part and methods of processing. However, in cases of plant poisoning, species and phytochemical characterization for crude recognition of likely toxins in relation to known plant toxins should be attempted. So, the present study investigated the toxicity of *G. elegans* Benth., followed by isolation and structural identification of toxic alkaloid compound. This plant, identified as *Gelsemium elegans* Benth. (နာကို / နာငါး), family of Loganiaceae is a well-known toxic indigenous plant of Southeastern Asia, used among hilltribes for committing suicide. Its leaves are morphologically similar to *Dregea volubilis* Benth. (ရွေးတောက်) and was mistaken by people at Mine-Khon Village, Kyin-ton Township who suffered strychnine-like toxic symptoms soon after ingestion. Toxicity studies were conducted using animal model and pure compound isolated by preparative thin layer chromatography (PTLC) was identified by UV, FT-IR, ^1H NMR and ^{13}C NMR spectrums. Acute toxicity study of watery extract of leaves and stems of *G. elegans* (400 mg/kg and 600 mg/kg) and total alkaloid fraction of leaves (100 mg/kg) showed lethal effect within 10 minutes, per orally in mice. Isolated pure alkaloid compound from total alkaloid fraction of leaves was identified to be 14-hydroxygelsedine ($\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_4$).

INTRODUCTION

Plant xenobiotics, as well as therapeutic ingredients can be organized using a pharmacognosy approach, which is known to be consistent with the literature of plant efficacy and toxicity. Alkaloids are one of the several classes of organic molecules found in plants that typically have strong pharmacologic activity that defines many major toxidromes. Plant chemistry is complex, containing multiple chemicals and chemical classes that work independently or in concert, rather than one toxin class per plant, per symptom group. Additionally, different plant families may contain similar, if not identical, xenobiotics which many of

them remained unidentified. Understanding of plant toxicity is poor relative to that of pharmaceutical agents since many are available only as case reports, lacking clear links between toxin exposure and illness, compounded by the fact that plant themselves are inherently variable, and the potency and type of toxin depend on the season, geography, local environment, plant part and methods of processing [1].

This plant, identified as *Gelsemium elegans* (နာကို၊ နာငါး), family of Loganiaceae is a well-known toxic indigenous plant of Southeastern Asia, used among hilltribes for committing suicide [2, 3]. Its leaves are morphologically similar to *Dregea volubilis*

Benth. (ခွေးတောက်) and were mistaken by people at Mine-Khon Village, Kyineta Township who suffered strychnine-like toxic symptoms soon after ingestion (Fig.1). Therefore, leaves and stems of *Gelsemium elegans* Benth. were undertaken to evaluate the acute toxicity, histopathological study and isolation of toxic alkaloid compound.



Gelsemium elegans Benth. *Dregea volubilis* Benth.

Fig. 1. Morphology of *Gelsemium elegans* Benth. and *Dregea volubilis* Benth.

Myanmar name: နာကို၊ နာငါး၊ နာငါး

Botanical name: *Gelsemium elegans* Benth.

Synonym: *Medica elegans* Gard & Champ.

Family name: Loganiaceae

Gelsemium is a small genus of three species, of which two species of *G. sempervirens* A. and *G. rankinii* S. are native to North America. *G. elegans* Benth. which is distributed in Southeast Asia, is very toxic and has been used traditionally for the treatment of pain, spasticity and skin ulcers in Chinese folk medicine [4]. In Myanmar, it is grown in Kachin State, upper Chindwin, Bhamo, Myitkyina, Mogoke and Wa State [5]. The whole plant is poisonous, in order of decreasing toxicity from root, leaves, flower, fruit to stem. Seventeen alkaloid monomers have been isolated in *G. elegans* Benth. Depressions of the nervous and respiratory systems are the main causes of death. It inhibits the respiratory center of the spinal cord resulting in respiratory depression and muscle paralysis [6].

Gelsemium elegans Benth. as an analgesic and the *Gelsemium* alkaloids act centrally against gamma amino butyric acid (GABA) action which result in clonic convulsion and

death [2]. *Gelsemium* alkaloids are classified into six types: sarpagine, koumine, humanenine, gelsedine, gelsemine and yohimbane. Of these, some gelsedine-type alkaloids showed potent cytotoxic activity against A431 epidermoid carcinoma cells [4]. Since it has been recommended that in cases of plant poisoning, species and phytochemical characterization for crude recognition of likely toxins in relation to known plant toxins should be attempted, leaves and stems of the *G. elegans* Benth. were evaluated for acute toxicity, and further subjected to histopathological study and isolation of toxic alkaloid compound from leaves.

Objective

This study was done to investigate the toxicity and to isolate toxic alkaloid compound from *G. elegans* Benth.

MATERIALS AND METHODS

The plant samples were collected from Kyineta area. Preliminary phytochemical investigation of plant materials (leaves and stems) was done by the method of Harborne [7]. Detected phytoconstituents of secondary metabolites (alkaloid) were extracted by Unani Formulation [8]. Dry plant samples were extracted with distilled water at 70°C by temperature control waterbath and the filtrate was evaporated to dried residue.

Extraction of total alkaloid fraction

Ten grams of leaves were extracted with 0.5N H₂SO₄ before being filtered. The filtrate was mixed with 30ml of chloroform and shaken for 30 minutes. The lower chloroform layer was collected using a separating funnel and the remaining upper acid layer was made alkaline by adding an excess of dilute NH₄OH and shaken for 20 minutes. After standing for 10 minutes, the chloroform layer was separated and later removed using a rotatory evaporator. The remaining dried residue was dehumidified in vacuum desiccators until a constant weight was obtained and the final weight

was taken as total alkaloid fraction [8]. This total alkaloid fraction was screened by thin layer chromatography (TLC) to isolate a pure indole alkaloid compound.

Toxicity study of plant samples

Acute oral toxicity test of watery extracts of leaves (400 mg/kg) and stems (600 mg/kg) was done according to Litchfield and Wilcoxin method [9]. Five animals of both sex of ddy strain healthy albino mice (28-35 gm) were used in each group. Total alkaloid fraction of leaves of *G. elegans* Benth. (25, 50, 100, 200 mg/kg) was used according to the OECD Guideline-423 [10]. In this study, three animals of single sex (females) of ddy strain healthy albino mice (28-35 gm) were used in one group. They were allowed free access to water and fed throughout the experiment.

All mice were subjected to gross necropsy. All gross pathological changes were recorded for each animal. For histopathological examination, selected five organs (livers, lungs, heart, kidney and brain) were collected and then fixed in 10% formalin. Paraffin sections were prepared and stained for microscopic examination at Pathology Department, University of Medicine 1, Yangon.

Isolation and identification of toxic compound

The isolation of indole alkaloid compound from total alkaloid fraction of *G. elegans* Benth. leaves were done by preparative thin layer chromatography (PTLC). Total alkaloid fraction was subjected to repeated chromatography by means of silica gel GF₂₅₄ TLC plate using chloroform: ethyl acetate: diethylamine (2:1:3) solvent system for separation. Purified toxic indole alkaloid compound was scraped from TLC plate, and then checked by UV light (254 nm) and spraying Dragendroff reagent [11, 12]. Characterization and identification of isolated pure indole alkaloid compound was performed by Ultraviolet spectrum, Fourier Transform Infrared spectrum and ¹H and ¹³C Nuclear Magnetic Resonance spectroscopy [13].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed the presence of alkaloids, glycosides, amino acid, steroids/ terpene, flavonoids, polyphenols, in leaves and stems of *G. elegans* Benth. and cyanogenic glycoside was absent in this plant. Alkaloid was found as major constituent in leaves and stems. All of the plants were digested primary metabolites (carbohydrate, amino acid, etc.) and produced secondary metabolites (alkaloid, flavonoids, polyphenols, triterpenes, etc.). These secondary metabolites have pharmacological effect and toxic effect. Alkaloids are often toxic to man and many have dramatic physiological activity [7]. Therefore, total alkaloid fraction was extracted from leaves, and then isolation of pure alkaloid compound from total alkaloid fraction of leaves was done.

Toxicity study

Acute oral toxicity results of watery extract of leaves (400 mg/kg), stems (600 mg/kg) and total alkaloid fraction of leaves (25, 50, 100, 200 mg/kg) are shown in Table 1.

Table 1. Results of acute toxicity test of *Gelsemium elegans* Benth. watery extract and total alkaloid fraction on mice

Plant samples	Dose (mg/kg)	Death (%)
Watery extract of leaves	400	100
Watery extract of stems	600	100
Total alkaloid fraction of leaves	200	100
Total alkaloid fraction of leaves	100	100
Total alkaloid fraction of leaves	50	0(no death)
Total alkaloid fraction of leaves	25	0(no death)

Test animals those died were seen within 10 minutes. The clinical signs and symptoms were observed as restlessness, excitation, hyperventilation, muscle contraction, piloerection, palpitation, finally respiratory depression and convulsion and muscle rigidity after death. According to OECD Guideline - 423, 2001, the oral LD₅₀ value of total alkaloid fraction of leaves determined in mice was cut-off value of 100 mg/kg body weight.

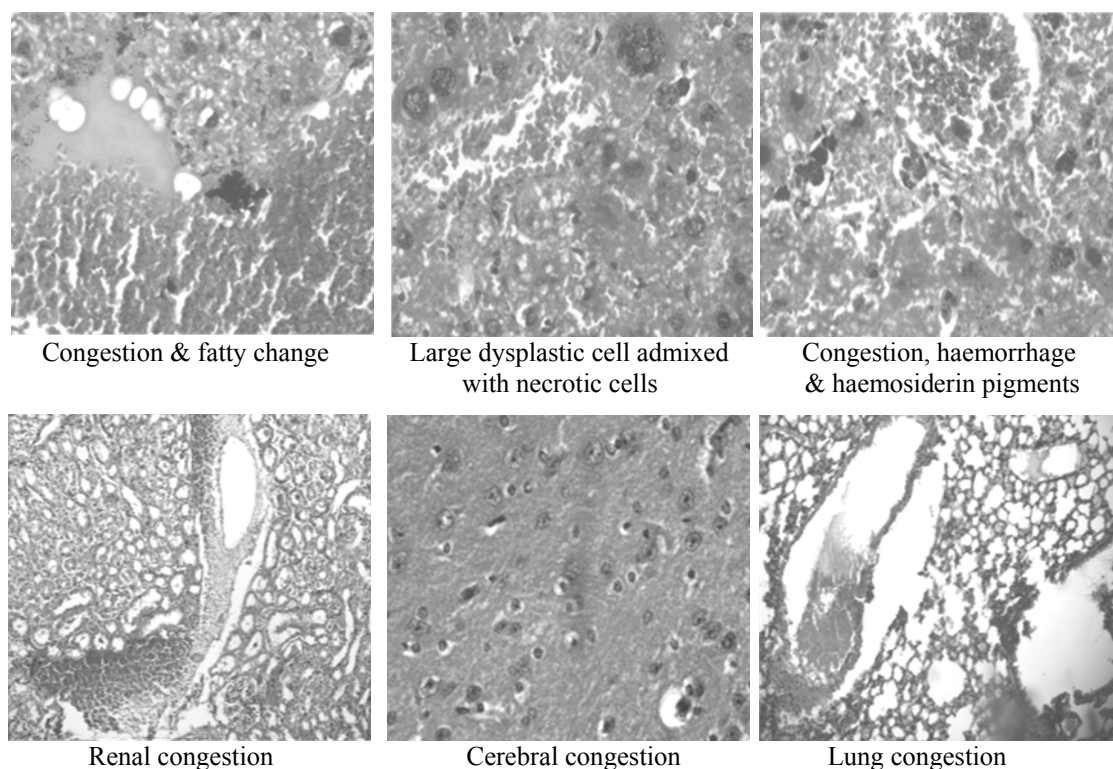


Fig. 2. Histopathological sections of some organs showing abnormalities in mice treated with total alkaloid fraction of *Gelsemium elegans* Benth.

Histopathological studies were as follows: Liver showed marked congestion of central veins, focal necrosis of hepatocytes 5-8 cells, fatty degeneration, severe dysplastic changes of hepatocytes, lymphocytic infiltration and massive centrilobular necrosis. Congestion, oedema of cerebrum and cerebellum were observed in brain. Congestion and interstitial pneumonitis were observed in lungs. Congestion and mesaginal proluxeration of glomeruli were observed in kidneys as shown in Fig. 2. It produced cytotoxic effects in the liver but heart tissue was normal.

Identification of isolated pure compound

Isolated pure compound (R_f value 0.6) from total alkaloid fraction of leaves (2.8%) was characterized by TLC screening. It showed orange color with Dragendroff spray and dark spot under UV-254 nm. Isolated compound identified by UV spectrum (255 nm, 278 nm) and FT-IR spectrum showed 3440 cm⁻¹ for -OH, 1728 cm⁻¹ for =NH & C=O, 1458 cm⁻¹ for -NH amide, 1388 cm⁻¹ for CH₂=CH₃. These data are in agreement

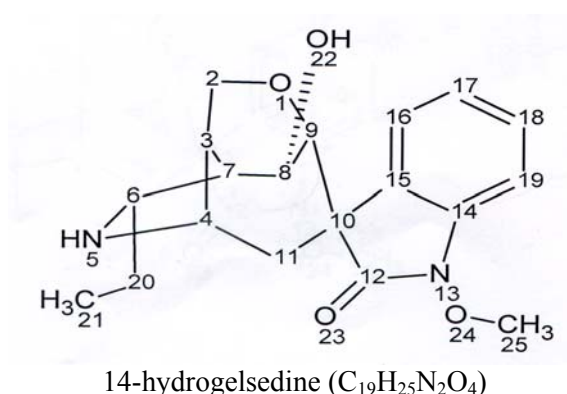


Fig. 3. Chemical structure of 14-hydrogelsedine (C₁₉H₂₅N₂O₄)

with literature [4, 14]. Therefore, isolated compound may be gelsedine type indole alkaloid. According to ¹HNMR and ¹³CNMR spectrums, isolated alkaloid compound contains nineteen protons and twenty-five carbons. ¹HNMR and ¹³CNMR spectral data of isolated compound compared with reference δC and δH value of ACD software are shown in Table 2.

Thus, the isolated alkaloid compound was confirmed as being 14-hydroxygelsedine (C₁₉H₂₅N₂O₄) (Fig. 3).

Table 2. ^{13}C and ^1H NMR spectra data of isolated compound from *G. elegans* Benth.

Carbon no.	δC (ppm)		H no.	δH (ppm)	
	Results	Ref. value		Results	Ref. value
C-2	61.6	61.98	H-2	2.3, 2.4	2.8, 2.7
C-6	60.9	61.31	H-7	2.64	2.7
C-8	67.4	66.22	H-8	4.5	4.15
C-9	77.9	77.47	H-16	7.2	7.49
C-12	174.1	175.02	H-17	7.18	6.97
C-14	140.2	138.75	H-21	0.86, 0.87	0.82, 0.84, 0.86
C-21	130.0	128.44	H-22, H-5	0.62	0.637
C-25	62.8	63.4	H-25	4.12	3.83

Conclusion

Ingestion of *Gelsemium elegans* Benth. is highly poisonous regarding its neurological and respiratory depressive effects. Prevention of *Gelsemium* poisoning is of paramount importance. People should be aware of plant poisoning and should avoid eating any wild plants because of similar external appearance between certain non-poisonous and poisonous species which can be fatal. Although plant analysis may not guarantee that all toxic mechanisms can be worked out, some reassurance can be achieved by excluding exposures to most life-threatening plant and to ascertain whether the exposure can be toxic.

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Usefulness of ascitic fluid cholesterol measurement in diagnosis of malignancy

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Ascitic fluid studies are fundamental not only for the effective care of the patients but also for establishing a reliable diagnostic test to identify the aetiology of diseases, both benign and malignant. From August 2006 to April 2008, a hospital and laboratory-based, cross-sectional analytical study was conducted at Yangon General Hospital, to find out the diagnostic accuracy of the ascitic fluid cholesterol analysis in diagnosis of malignant ascites. A total sample size of 60 samples of ascitic fluid was involved in this study. For all cases, ascitic fluid cholesterol level was determined by using Bayer Express Plus Auto Analyzer. Cytological investigation was employed as the standard diagnostic tool. The mean value of ascitic fluid cholesterol level of malignant source (2.26 ± 0.48 mmol/l) was statistically higher than that of non-malignant one (1.24 ± 0.59 mmol/l) ($p < 0.05$). Moreover, the cutoff point of the ascitic fluid cholesterol level (1.7 mmol/l) was preferred by using Receiver Operating Characteristics Curve (ROC Curve) and the sensitivity, specificity and accuracy of this biochemical parameter to differentiate either benign or malignant diseases were 85.7%, 87.5% and 86.6%, respectively. Taking into consideration the above results, persistent elevation of ascitic fluid cholesterol level above the cutoff value should alarm the suspicion of malignant aetiology. For that reason, the ascitic fluid cholesterol measurement could be applied as a potential indicator of carcinomatosis in clinical practice.

INTRODUCTION

Ascites is the pathologic accumulation of fluid in the peritoneal cavity and it is a common clinical finding with a wide range of causes. The differential diagnosis of ascites is a lengthy one for clinicians. As a result, ascitic fluid study is vital for the effective care of the patients as well as fundamental to find out a reliable test for identifying the aetiology of disease either benign or malignant. Although various diagnostic tools including radiological and laboratory investigations are required to get the correct diagnosis for the effective treatment of the patients, potential biological indicator for prediction of malignancy is still needed in clinical practice. Moreover, many biological markers such as protein, sugar, lipid (cholesterol, low density lipoprotein, apolipoprotein-A1, and apolipoprotein-B), enzymes (lactate de-

hydrogenase, amylase), trace elements (zinc, magnesium), tumour markers (carcino-embryonic antigen (CEA), prostatic specific antigen (PSA)), cytokines, fibronectin and sialic acid, are applied as the markers for identifying the aetiology of the disease.

Among them, lipid estimation is a simple biochemical analysis and it can be applied at the poor resource center. After that, lipids are also essential in intermediary metabolism, principally as a major source of metabolic fuel and as structural components of cell membranes. By using this simple diagnostic tool, ascitic fluid cholesterol level, the underlying origin of ascites, either benign or malignant, can be diagnosed for patients with inconclusive and negative cytology. Researchers discovered that ascitic fluid cholesterol level is useful to distinguish the malignant and non-malignant ascites and is also a valuable supplement to

cytologic examination in distinguishing between ascites of malignant and benign origin [1, 2]. By making use of a simple biochemical parameter as a potential predictor of malignancy, this study helps the clinicians for the better care of patients. The aim of this study was to find out the diagnostic accuracy of the ascitic fluid cholesterol analysis in diagnosis of malignant ascites.

MATERIALS AND METHODS

This cross-sectional analytical study which is hospital and laboratory-based, was done at the Pathology Department, Yangon General Hospital during the period August 2006 to April 2008. Total 60 cases of ascites samples were involved in this study and systematic sampling procedure was used for the selection of samples. Regarding the ethical consideration, this study was approved by the (Academic and Ethic) Board of University of Medicine 1, Yangon.

Cytological examination of ascitic fluid and determination of ascitic fluid cholesterol level were performed to all the samples and then correlated with final diagnosis. Ascitic fluid cholesterol level was determined by using the enzymatic end point method {Cholesterolo 4 (Hospitex Diagnostic)} at 546 nm wavelength with Bayer Express Plus Auto Analyzer. The principle of the test was that the cholesterol was determined after enzymatic hydrolysis and oxidation and the indicator quinoneimine is formed from hydrogenperoxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Cytological investigation was employed as the standard diagnostic tool in this study. Regarding the procedure of the cytology, firstly the ascitic fluid was centrifuged at the speed of 2000 rpm for 20 minutes and sediment was spread on glass slide and fixed in the solution of absolute alcohol for 30 minutes. Then slides were stained with Papanicolaou method. Cytological examination was done by principal investigator and counterchecked by the Senior Consultant Pathologist. All the

laboratory experiments were done according to the quality control steps and procedures.

Data entry was done by using Microsoft Office Excel and analysis was done by using SPSS software 13.0 version. Frequency charts were defined and continuous variables' averages and standard deviations (SD) were calculated. Statistical significance tests were used when making comparisons, if necessary (Student's t , χ^2), having a <0.05 acceptance level.

RESULTS

During the study period, 60 cases of ascitic fluid samples, 29 cases (male) and 31 cases (female) were analyzed in this study. Patients with ascites, 32 cases (53%) of benign and 28 cases (47%) of malignant origin, were included in this study. Among the non-malignant cases, 27 samples were due to cirrhosis and five numbers were due to tuberculosis. The mean ascitic fluid cholesterol level of benign diseases was 1.24 mmol/l (SD-0.59) and for the malignant source was 2.26 mmol/l (SD-0.48). The mean value of ascitic fluid cholesterol levels in malignant cases was significantly higher than that of benign aetiology ($p=<0.05$). By the use of the statistical software SPSS 13.0 version, Receiver Operating Characteristics Curve (ROC Curve) was applied for identifying the best cutoff point for ascitic fluid cholesterol level (mmol/l) to differentiate between benign and malignant ascites (Fig.1.).

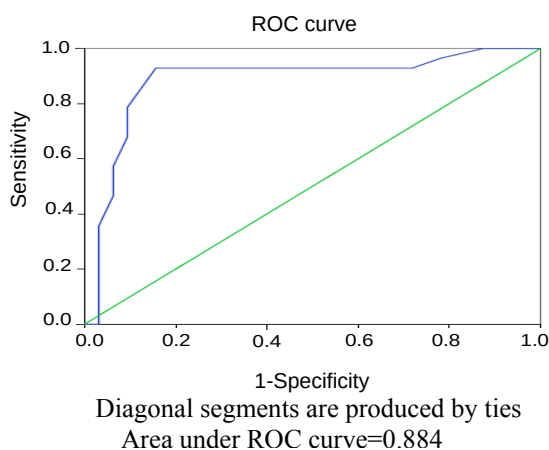


Fig.1. Receiver Operating Characteristics curve for ascitic fluid cholesterol level

The sensitivity and specificity of the different levels of ascitic fluid cholesterol are shown in Table 1 as follow.

Table 1. The sensitivity and specificity of the different levels of ascitic fluid cholesterol levels

Ascitic fluid cholesterol level (mmol/l)	Sensitivity (%)	Specificity (%)
0.9	100	13.5
1.0	92.2	28.1
1.1	92.9	34.4
1.2	92.9	43.7
1.3	92.9	59.4
1.4	92.9	75.0
1.5	92.9	84.4
1.6	86.7	85.5
1.7	85.7	87.5
1.8	78.6	90.6
1.9	67.9	90.6
2.0	57.1	94.7
2.2	53.6	94.7

According to the above results, the best cut-off point for the diagnosis of malignancy was 1.7 mmol/l and ascitic cholesterol level more than or equal to 1.7 mmol/l expressed malignant ascites and less than 1.7 mmol/l was benign one. However, the ascitic fluid cholesterol levels for four malignant samples were false negative results, below the cutoff point, 1.7 mmol/l, and four benign cases also showed false positive results, above the cutoff point. So, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 85.7%, 87.5%, 85.7%, 87.5% and 86.6%, respectively.

DISCUSSION

In the present study, the mean ascitic fluid cholesterol level for non-malignant ascites was 1.24 mmol/l and for malignant cases was 2.26 mmol/l. Thus, the mean ascitic fluid cholesterol level was significantly higher in malignant source than in benign one in this study ($p < 0.05$). The previous studies reported that the mean ascitic fluid cholesterol level for non-malignant disease was 0.8 mmol/l and for malignant one was 1.1 mmol/l, respectively [3]. Moreover, a study in India had observed that the mean ascitic fluid cholesterol level for the cases of

ascites due to cirrhosis was 0.8 mmol/l and for the cases of ascites due to malignancy was 2.5 mmol/l [4]. Ascitic fluid cholesterol estimation is a reliable and simple test for differentiating malignant origin from tuberculous ascites. One study also described that mean ascitic cholesterol level was significantly higher in malignant diseases (89.52 mg/dl) as compared to ascites due to tuberculosis (35.07 mg/dl) [5]. Therefore, all these data highlight the usefulness of ascitic fluid cholesterol estimation in differentiating malignant ascites from non-malignant one and the source of cholesterol in ascitic fluid was the malignant cell membranes.

Using the SPSS software, the cutoff value of the diagnostic test evaluation parameter for differentiating between benign and malignant cases was chosen by drawing ROC curve. According to this evaluative curve, if the ascitic fluid cholesterol level (1.5 mmol/l) was chosen as a cutoff value to differentiate the two origins of the diseases, sensitivity of the test was 92.9% and specificity was 84.4%. For this cutoff value, the sensitivity of the test was good but the specificity was poor. When ascitic fluid cholesterol level (1.8 mmol/l) was preferred as cutoff point, the sensitivity was 78.6% and specificity was 90.6%. At this cutoff value, the sensitivity was reduced but the specificity was superior. If the cholesterol level (1.7 mmol/l) was selected, the sensitivity was 85.7% and specificity was 87.5%. Taking into consideration the above results, the ascitic fluid cholesterol level, 1.7 mmol/l, was the reliable value for identification of aetiology of the ascites, either benign or malignant one.

Regarding the diagnostic accuracy of this biological marker, the accuracy of the ascitic fluid cholesterol level to detect benign and malignant cases was 86.6%, sensitivity was 85.7%, specificity was 87.5%, positive predictive value was 85.7% and negative predictive value was 87.5% in this study. In one study, ascitic fluid cholesterol level, more than 1.7 mmol/l, was

used as cutoff level to identify malignancy and the sensitivity, specificity, positive predictive value and negative predictive value were 96%, 96%, 96% and 96%, respectively [4]. Therefore, the cutoff value was more or less similar with this study.

The preceding studies reported that ascitic fluid cholesterol estimation had the best Youden Index (57%), sensitivity 78.3% and specificity 79.3% at a cutoff value, 0.9 mmol/l, by using the ROC [6]. It was also described that ascitic cholesterol measurement is a valuable supplement to cytologic examination in distinguishing between ascites of malignant and benign origin. In this current study, if the cutoff value, 0.9 mmol/l, was chosen, the sensitivity and specificity of the test were 100% and 13.5%, respectively. So, the sensitivity of the test was good but the specificity was very low. As a result, this cutoff value was not consistent in this study. The diagnostic value of an ascitic fluid cholesterol concentration less than or equal to 1.2 mmol/l in terms of benign diseases was 80.0% and greater than 1.2 mmol/l in terms of predicting malignant diseases was 87.5% [1]. Some authors also stated that ascitic fluid cholesterol value, more than 1.21 mmol/l, is a sensitive marker for carcinomatosis [7]. In this study, 1.2 mmol/l was used as the cutoff value for ascitic fluid cholesterol level and, the sensitivity was 92.9%, and specificity was 43.7%. As a result, this study agrees with the findings of Mortensen, *et al*, and Gulyas, *et al* [1, 7].

A study reported that the mean ascitic cholesterol level was significantly higher in malignant ascites than in non-malignant ascites and (88%) patients with malignant one could be separated from the latter. After that, the specificity (100%) and diagnostic efficiency (94%) of ascitic fluid cholesterol was better than with (84%) specificity and (86%) diagnostic efficiency that of serum ascitic albumin gradient [8].

The raised ascitic fluid cholesterol level in the malignant aetiology was observed in this study. However, the origin of increased

cholesterol in ascitic fluid in this study could not be explored and association between level of hypercholesterolaemia and the histological types of malignant tumours as well as organ specificity of malignancy were not identified in this current study. It can be made possible by using advanced diagnostic methods and experimental studies. As a conclusion, increased ascitic fluid cholesterol level in a cytologically benign patient helps the clinicians to be aware of the possible malignancy in the causation of ascites.

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**Bacteriological profile of surgical site infections
in gynaecological ward of North Okkalapa General Hospital**

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Surgical site infection (SSI) is one of the most common post-operative complications of the gynaecological surgeries. A cross-sectional descriptive study was conducted in gynaecological ward of North Okkalapa General Hospital from January to October 2010 to identify the causal pathogenic bacteria and to determine antibiotic susceptibility pattern. Wound swab specimens were collected from 43 SSI cases to perform standard laboratory tests at Bacteriology Research Division, DMR (LM). Out of 25 culture-positive cases, 28 aerobic bacterial pathogens; ie., *Staphylococcus aureus* (14/28, 50%), *Pseudomonas* species (8/28, 28.57%), coagulase-negative staphylococci (3/28, 10.71%), *Escherichia coli* (2/28, 6.14%) and *Enterococcus* species (1/28, 3.57%) were isolated. Amikacin and cefoperazone/sulbactam were found to be the most sensitive antibiotics for gram-positive and gram-negative bacteria, respectively. The research findings provided the necessary data for case management and formulation of appropriate antibiotic regimen for the respective hospital.

INTRODUCTION

Surgical site infection (SSI) is one of the most common complications after gynaecological operations. It is associated with considerable financial and social problems for the family and also makes heavy demands on scarce hospital beds. In many countries, gynaecological surgical site infection rate is found to be the highest among other post-operative complications ranging from 2.26-12.17% [1, 2, 3]. In Myanmar, there is limited published data of SSIs after gynaecological operations although many SSIs studies had done on general and other specialty surgeries. In the study of post-operative surgical wound sepsis of clean elective orthopaedic cases in the orthopaedic and trauma unit of North Okkalapa General Hospital (NOGH), the overall post-operative infection rate was 6.7% [4]. Prevention and proper treatment of surgical site infections with appropriate

antibiotics is a primary management objective for all healthcare practitioners.

Surgical site infections may be due to endogenous flora or acquired hospital infections and surgical site infections rate varies from one hospital to another relating to a member of operating team or to some other environmental sources in the operating theater [5]. It also depends upon wound type, length of hospital stay, surgical procedure, pre-operative condition of the patient and the presence of various risk factors. Type of bacteria found in surgical site infections also varies with geographical location, bacteria resident on the skin at the site of wound [6].

Moreover, the emergence of drug-resistant bacteria plays an important role in rising wound infection rate and treatment failure cases. It is also important to determine antibiotic sensitivity pattern of pathogenic bacteria to currently using antibiotics.

Information about the bacterial pathogens and antibiotic sensitivity will help to improve patient care and save money by selecting the most suitable antibiotics. Knowledge of sensitivity pattern of the hospital flora is critical to antibiotic selection.

The present study was carried out to identify pathogenic bacteria from surgical site infections in gynaecological ward of NOGH and to determine the antibiotic sensitivity pattern of the isolated bacterial pathogens and to find out associated factors for SSIs among study population.

MATERIALS AND METHODS

A cross-sectional descriptive study was carried out from January to October 2010 on 43 patients admitted to the gynaecological ward of NOGH with SSIs after undergoing gynaecological surgery. Surgical site infection is clinically detected according to Centers for Disease Control and Prevention (CDC) criteria [7].

Wound swab specimens were collected after taking the informed consent. Demographic and clinical data were taken in the proformer. The specimens were inoculated into Stuart transport medium and sent to Bacteriology Research Division, Department of Medical Research (Lower Myanmar) to perform laboratory tests.

The specimens were inoculated onto blood agar, MacConkey agar, and nutrient agar plates. Blood agar plate was incubated in 37°C incubator under 5-10% CO₂ concentration and MacConkey and nutrient agar plates were incubated at 37°C for overnight. Isolated colonies were identified macroscopically and microscopically (gram staining), and by a series of biochemical tests according to Cowan & Steel [8] and Ewing [9].

Antimicrobial susceptibility test for each isolated pathogen was performed on a Mueller-Hinton agar plate by modified disc diffusion technique of Kirby-Bauer [10]. E test was used to detect MICs of penicillin and amoxicillin/clavulanic acid on most

frequently isolated gram-positive bacteria (*Staphylococcus aureus*) and ciprofloxacin and ceftriaxone on most frequently isolated gram-negative bacteria (*Pseudomonas* species).

Data were checked for completeness, errors and inconsistencies, prior to data entry and analysis was done by statistical Software SPSS 15.

RESULTS

During the study period, a total of 43 SSI cases were detected clinically. Among them, two types of post-operative wound sepsis were observed: superficial incisional SSIs (n=41) and deep incisional SSIs (n=2). Culture positivity was 58.14% (25/43) comprising 56.1% (23/41) in superficial incisional SSIs and 100% (2/2) in deep incisional SSIs.

Surgical site infections in relation to type of surgical wound

Out of 43 cases of clinically detected SSIs, bacterial pathogens were isolated in 50% (17/34) of clean-contaminated and contaminated procedures and 88.9% (8/9) of dirty procedures. Dirty wounds were significantly more likely to have culture-positive result than clean-contaminated and contaminated procedures (Crude OR=8.0; 95% CI=0.9-71.1; p=0.05).

SSIs in emergency and elective operations

Bacterial pathogens were isolated in 9 out of 10 (90%) of emergency cases and 16 out of 33 (48.5%) of elective cases. SSIs after emergency operations were significantly more likely to have culture-positive result than elective operations (Crude OR=9.5; 95% CI=1.1- 84.2; p=0.02).

SSIs in relation to type of suturing materials

Bacterial pathogens were isolated in 10/11 (90.9%) of SSI cases using thread to close skin and 15/32 (46.5%) of cases using other materials. Use of thread to close skin on procedures was significantly more likely to have culture-positive result than use of other

materials (Crude OR=11.0; 95% CI=1.3-99.2; p=0.01).

Surgical site infections in relation to predisposing factors

Presence of predisposing factors was found in 74.4% (23/43) of the cases. The most common predisposing factor was found to be anaemia (n=26), followed by malignancy (n=12), presence of infection (n=9), old age (60+) (n=5), diabetes (n=2), and immuno-suppressive therapy (n=1). There is a significant association between culture positivity and patients presenting with combined predisposing factors (p=0.02) (Table 1).

Table 1. Surgical site infections in relation to predisposing factors

Predisposing factors	Clinically detected SSIs				Total	
	Culture					
	positive		Negative			
	No.	%	No.	%	No.	%
Without predisposing factor	6	54.5	5	45.5	11	100
With single factor	3	25	9	75	12	100
With two factors	13	81.3	3	18.8	16	100
With three factors	3	75	1	25	4	100
Total	25	58.1	18	41.9	43	100

(p=0.02)

Types of bacterial pathogens isolated

Types and frequency of bacterial pathogens isolated from 43 SSI cases are presented in Table 2.

Antimicrobial susceptibility pattern

The most common isolate, *Staphylococcus aureus* was found to be 92.31% sensitive to amikacin (12/13). Second highly sensitive antimicrobial drug was lincomycin (11/13, 84.81%). It was moderately sensitive to cefoperazone/sulbactam (9/13, 69.32%), cefepime (8/13, 61.54%), piperacillin/tazobactam and amoxicillin/clavulanic acid (7/13, 53.85% each) and mildly sensitive to ceftazidime, sulphamethoxazole/trimethoprim, ofloxacin and oxacillin (6/13, 46.15% each), gentamicin (5/13, 38.46%) and ciprofloxacin (4/13, 30.76%) and poorly

sensitive to ceftriaxone (3/13, 23.08%) and penicillin (2/13, 15.38%).

Table 2. Types of isolated bacterial pathogens

No.	Bacteria isolated	Single infection	Mixed infection	Frequency of culture positive No. (%)
1.	<i>Staph. aureus</i>	13	1	14(50)
2.	<i>Pseudomonas spp.</i>	5	3	8(28.57)
3.	Coagulase-neg Staph.	3	0	3(10.71)
4.	<i>Escherichia coli</i>	1	1	2(7.14)
5.	<i>Enterococcus spp.</i>	0	1	1(3.57)
Total		22	6	28(100)

Among 7 methicillin-resistant *Staphylococcus aureus* (MRSA), 1 isolate was found to be resistant to vancomycin. MRSA were totally (100%) resistant to ceftriaxone. Six out of 7 (85.7%) of MRSA were resistant to ceftazidime and gentamicin and piperacillin/tazobactam.

For *Pseudomonas* species, cefoperazone/sulbactam was the most sensitive drug (8/8, 100%) followed by piperacillin/tazobactam (7/8, 87.5%) and amikacin, cefepime, ceftazidime (6/8, 75% each). It was 37.5% (3/8) each sensitive to ciprofloxacin, gentamicin, ofloxacin and sulphamethoxazole/trimethoprim. It showed low sensitivity to amoxicillin/clavulanic acid (1/8, 12.5%) and totally resistant to ceftriaxone and lincomycin (0/8, 0%). One isolate showed sensitive only to cefoperazone/sulbactam and resistant to all other drugs. Another one was sensitive to two drugs, cefoperazone/sulbactam and piperacillin/tazobactam.

Antibiotic susceptibility of coagulase-negative staphylococci showed 100% (3/3) sensitive to amikacin, 66.67% (2/3) each sensitive to cefepime, cefoperazone/sulbactam, ceftazidime, gentamicin, lincomycin, ofloxacin, piperacillin/tazobactam and sulphamethoxazole/trimethoprim, 33.33% (1/3) each sensitive to ciprofloxacin and oxacillin, and totally resistant to amoxicillin/clavulanic acid and penicillin. One isolate of coagulase-negative staphylococcus was resistant to vancomycin.

The two isolates of *Escherichia coli* were 100% sensitive to amikacin, cefepime, cefoperazone/sulbactam, ceftazidime and piperacillin/tazobactam, 50% (1/2) sensitive to ofloxacin and lincomycin, and totally resistant to amoxicillin/clavulanic acid, ceftriaxone, ciprofloxacin, gentamicin and sulphamethoxazole/trimethoprim. One isolate of *Enterococcus* species was only sensitive to amoxicillin/clavulanic acid and resistant to all other drugs including vancomycin.

E test for detection of MIC

The two most isolated organisms *Staphylococcus aureus* and *Pseudomonas* species were tested for MIC by E test strips. Thirteen isolates of *Staphylococcus aureus* were tested for MIC of penicillin and amoxi-cillin/clavulanic acid and 8 isolates of *Pseudomonas* species were tested for MIC of ceftriaxone and ciprofloxacin. MICs of chosen drugs for *Staphylococcus aureus* and *Pseudomonas* species are presented in Table 3.

Table 3. MIC of antibiotics on isolated bacteria

Organism	Antibiotics	Susceptibility	No. of isolates	MIC values µg/ml
<i>Staph. aureus</i>	Amoxicillin/clavulanic acid	sensitive	7	0.064-4
		resistant	6	24-48
	Penicillin	sensitive	2	0.064-0.094
		resistant	11	0.125- >32
<i>Pseudo-monas</i> species	Ceftriaxone	sensitive	0	-
		Intermediate	0	-
		resistant	8	>32
	Ciprofloxacin	sensitive	3	0.19-0.38
		Intermediate	1	3
		resistant	4	>32

Antimicrobial susceptibility pattern of bacterial pathogens to drugs used for prophylaxis treatment

All cases were given antibiotics before operation. Out of 43 cases, 29 cases were given as prophylaxis 1 hour before operation and 14 cases were given as curative treatment. The most commonly used antibiotics as prophylaxis before surgery was third-generation cephalosporin, ceftriaxone (28/29, 96.55%). The other drug was amoxicillin/clavulanic acid (1/29, 3.44%).

Among them, 79.31% (23/29) of cases were given combined with metronidazole.

Among 27 isolates that could be tested for antibiotics susceptibility, 18 numbers were resistant to ceftriaxone (66.67%), 5 were intermediate (18.52%) and only 4 (14.81%) were sensitive. Among the 27 isolates tested for amoxicillin/clavulanic acid, 17 (62.96%) isolates showed resistant and 10 (37.04%) were sensitive to that drug.

DISCUSSION

In the present study, a total of 43 SSIs cases including 41 cases of superficial incisional SSIs (95.35%) and two deep incisional surgical site infections (4.65%) were detected following different types of gynaecological operations. Deep-seated sepsis developing a few days after an operation and before the wound has been dressed reflects a theatre infection. Ward infections tend to be more superficial and frequently follow the dressing of wounds in the ward [11].

In the present study, the greater proportion of superficial SSIs indicates source of infection may be from hospital wards. However, it may be due to the fact that there is sometime difficult to assess extent of a wound as superficial or deep clinically. Though superficial incisional SSIs are vivid, deep incisional SSIs can not be assessed easily. Culture positive rate was 58.1% (25/43). However, culture-negative cases cannot be ruled out of SSIs because anaerobic organisms were not identified in this study.

Those who had dirty wounds, undergone emergency operations and the use of thread for skin closure were significantly more likely to get culture positivity than those without (Crude OR=8.0; 95% CI=0.9-71.1; p=0.05; crude OR=9.5; 95% CI=1.1-84.2; p=0.02; crude OR=11.0; 95% CI=1.3- 99.2; p=0.01). These findings were consistent with findings of other study [1].

More than two third of the clinically detected SSIs had single or multiple

predisposing factors. There is a significant association between culture positivity and presence of combined predisposing factors ($p=0.02$). It can be observed that each of above risk factors may cause impair immunity of the patient. So, the combination of two or more of these factors with inevitable surgical stress results in immunosuppression and development of any infection including SSI.

In the present study, 28 numbers of bacterial pathogens were isolated from 25 culture-positive cases. *Staphylococcus aureus* was the commonest isolate and the result was consistent with other studies of SSIs [3, 2-15]. Coagulase-negative staphylococci (CoNS), often previously dismissed as culture contaminants, are increasingly recognized as important nosocomial pathogens. In this study, 10.71% (3/28) of isolates were CoNS. *Pseudomonas* species were second most frequently isolated organisms in other SSIs studies [14-15].

Also in this study, *Pseudomonas* species was the second most isolated pathogen presenting 28.57% of all isolated pathogenic organisms. In the present study, 2 isolates of *Escherichia coli* were detected. As that organism is related to gastrointestinal tract, only few isolates could be detected in gynaecological surgeries. One and only isolate of *Enterococcus* species occurred as a mix infection with *Pseudomonas* species. Enterococci are normal inhabitants of the gastrointestinal tract and cause nosocomial urinary tract, blood stream, wound and intra-abdominal infections [16].

In this study, it was isolated from the patient who had undergone bowel surgery together with gynaecological operation. Thus, the source of enterococci may be from endogenous intestinal flora. As there are rapidly emerging nosocomial pathogens and the problem of multi-drug resistance, methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative pathogens expressing broad spectrum β -lactamases have already emerged. Among all National Nosocomial Infection Surveillance (NNIS) System

hospitals, MRSA rose from 2.4% in 1975 to 29% in 1991. MRSA is affecting patients in hospitals of all sizes and appears to be increasing [17]. In the studies of SSIs done in Myanmar, MRSA in post-operative wound infections were 13 - 72% [15-19].

In the present study, 53.85% of detected isolates were MRSA. One out of 7 of MRSA was found to be vancomycin resistant. Increasing MRSA infection may lead to the continuous use of vancomycin in the hospital and that in turn promote the growth of vancomycin-resistant organisms such as vancomycin-resistant enterococci and staphylococci. Most of the MRSA were multi-drug resistant. More than seventy five percent of MRSA were resistant to commonly used antistaphylococcus anti-microbials [18]. Also in this study, MRSA were found to be resistant to ceftriaxone (7/7, 100%), ceftazidime, gentamicin and piperacillin/ tazobactam (6/7, 85.7% each). All 3 isolates of CoNS found in this study were totally sensitive to amikacin (100%). One out of 3 (33.3%) was sensitive to oxacillin and ciprofloxacin, and it was totally resistant to amoxicillin/ clavulanic acid and penicillin. There was one vancomycin-resistant CoNS. According to some reports *Pseudomonas aeruginosa* showed a high sensitivity of 85% and 100% to penicillin derivatives (piperacillin/ tazobactam) [19, 20].

In the present study, cefoperazone/ sulbactam was the most sensitive drug for *Pseudomonas* species (8/8, 100%) followed by piperacillin/tazobactam (7/8, 87.5%). In one study, all strains of pathogenic *Escherichia coli* showed 100% sensitivity to penicillin derivatives (piperacillin/tazobactam) [19]. The result is consistent with that of the present study in sensitivity to piperacillin/tazobactam (2/2, 100%). Other sensitive drugs found in this study were amikacin, cefepime, cefoperazone/sulbactam and ceftazidime which also showed 100% sensitive.

Enterococci are intrinsically resistant to multiple antibiotics. Vancomycin-resistant

enterococci (VRE) are emerging pathogens that cause increasing proportion of nosocomial enterococcal infections. NNIS reports a 20-fold increase in VRE nosocomial infections from 1989-1993 [16]. One isolate of enterococcus found in the present study was vancomycin resistant.

One study showed that a significant portion of pathogens causing post-hysterectomy abdominal wall wound infection were resistant to the prophylactic treatment [21]. Also in present study, 27 out of 28 isolated bacteria were performed antibiotic susceptibility tests and most of the isolated bacteria (18/27, 66.67%) were resistant to the most frequently used drug, ceftriaxone and 18.52% (5/27) were intermediate resistant. Only 14.81% (4/27) were sensitive. MIC of ceftriaxone on *Pseudomonas spp.* also showed very high MIC (>32 µg/ml) and there was no sensitive strain at all.

Conclusion

A significant portion of the pathogens causing SSIs infection after gynaecological operations were resistant to the currently used prophylactic antibiotics. A high percentage of MRSA and MRCoNS with vancomycin-resistant strains including VRE were detected. Presence of multidrug-resistant strains including vancomycin-resistant strains in the ward is an alarming sign of spread and outbreak. Sensitivity of antibiotics that is commonly used for prophylaxis is very low on both gram-positive and gram-negative pathogens. So it is a need to evaluate the choice of antibiotics for prophylaxis and empirical therapy of SSIs in respective hospital.

The emerging resistance in nosocomial bacterial pathogens against commonly used antibiotics as well as some higher generation antibiotics such as vancomycin necessitates strict surveillance of these organisms, institution of effective infection control policies and judicious use of antibiotics. The research findings provided the necessary data for case management and formulation of appropriate antibiotic regimen of the respective hospital.

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Community-based control of *Aedes aegypti* larvae by using *Toxorhynchites* larvae in selected townships of Yangon Division, Myanmar

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A longitudinal community-based study was conducted to study the effect of *Toxorhynchites splendens* (Wiedemann 1819) (elephant mosquito) larvae on *Aedes aegypti* immature stages as biological control. Its effectiveness, acceptability, feasibility and cost effectiveness were determined in water storage containers of randomly selected 50 households from Shwepyitha Township as test area and untreated containers of 50 households from Dagon Myothit (North) as control area, respectively. Before intervention, *Aedes* larvae positivity in all kinds of water storage containers was examined and recorded. During intervention, second stage two elephant mosquito larvae each were put into all water storage containers bi monthly and larval positivity was recorded monthly for 3 months (before, during and after intervention month). Before and after intervention, House Index (HI), Container Index (CI), Breteau Index (BI) and percentage of reduction of key containers and positive premises were calculated. Initially in laboratory, maximum number of larval consuming rate was measured using *Aedes* and *Culex* larvae as prey to control mosquitoes population by *Toxorhynchites* larvae. After intervention, House Index, Container Index and Breteau Index were significantly reduced from 92%, 36.4% and 356 to 4%, 0.62% and 6, respectively, within 3 months. Also 93.75% reduction in number of key containers (>500 larvae positive/ container) and 95.24% reduction in positive premises (3 and above containers positive with larvae/house) were found in test area of Shwepyitha Township. The study indicated that *Toxorhynchites splendens* are very effective biocontrol agents against *Aedes* larvae of domestic water containers. The method is simple, acceptable, and cost effective and can be done by all health workers and household persons seasonally. It could be used to control *Ae. aegypti* adult density to prevent DF/DHF endemicity.

INTRODUCTION

Dengue fever/ dengue haemorrhagic fever (DF/ DHF) is the most important emerging viral disease and increasing the risk of DHF epidemics through the introduction of new serotypes. Globally, 2.5 billion people live in areas where dengue viruses can be transmitted: an estimated 50 million dengue infections occur each year, with 500,000 cases of DHF and at least 22,000 deaths

mainly among children [1]. The disease is transmitted by the vector *Aedes aegypti* mosquito. It can be reduced through destroying the *Aedes* immature stage by using biological method. *Aedes aegypti* is considered the major vector of dengue/ fever (DF) and dengue haemorrhagic fever (DHF) in Myanmar. DHF epidemic was reported for the first time in 1970 with the first major epidemic outbreak in Yangon [2]. Annual report of Vector Borne Diseases

Control (VBDC) revealed that 4759 DHF cases and 54 deaths (cases fatality rate= 1.13%) were found in Yangon Division, in 2007. About 60% of the DHF cases occurred in the 5-10 years old age group [3].

The rapid growth of population and industrial installation in urban and semi-urban areas has resulted in an alarming increase in mosquito's density. Untreated storing water in containers originated from pipe supply, tube wells, surface wells, ponds and rain water also encourage mosquito density. Mosquitoes are also becoming increasingly resistant to chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products [4]. Hence, it is imperative that novel mosquito control methods are developed and put into general use as soon as possible.

One potential alternative approach to the use of chemical pesticides is the use of *Toxorhynchites splendens* (Wiedemann 1819) (elephant mosquito) larvae as biological control agents to vector mosquitoes. *Toxorhynchites* spp. mosquitoes are recognized as potential biological control agents of vector mosquitoes. There have been many attempts to use them for this purpose since the beginning of the twentieth century. This was suggested as early as 1911 by W.R. Colledge (Royal Society of Queensland) [5].

Toxorhynchites mosquitoes have an unusual life cycle in that they are not capable of blood feeding and, therefore, are not vectors. In addition, their larvae are predatory on other mosquito larvae. *Toxorhynchites* spp. adult females seek out these same aquatic habitats to lay their eggs (both predator and prey may be attracted to the same oviposition sites) [6].

Therefore, a longitudinal community-based study was aimed to study the affect of *Toxorhynchites splendens* larvae on *Aedes aegypti* immature stages as biological control in selected areas of Yangon Division, Myanmar.

MATERIALS AND METHODS

Study areas

Fifty randomly selected households from Myoma Ward, Shwepyitha Township as test area and another 50 randomly selected households from 31st Ward, Dagon Myothit (North) Township as control area were involved in the study.

Study period and study design

Both laboratory and field study were done from June 2007 to September 2008 and study design was laboratory and field-based experimental descriptive study.

Collection of elephant mosquito larvae

Elephant mosquito larvae collection was done in Shwepyitha Township in all major (metal drum, cement tank, concrete jar, plastic drum, bago jar etc.), minor (earthen pot, small bago jar, plastic bowl etc.) and miscellaneous (discarded tire, batteries, small earthen pot, tin can, plastic bottle, coconut shell, bamboo tray, flower vases etc.) water containers. Positivity of elephant mosquito larvae was noted for its seasonal pattern. Collected elephant mosquitoes larvae were put into plastic bag and carried to Medical Entomology Section, NHL for further research.

Species identification

Collected elephant mosquitoes larvae were reared in laboratory till emerged to adult. Adult emerged elephant mosquitoes from survey were identified according to the key of Cheong [7] and Barraud [8].

Mass rearing

Collected elephant mosquito larvae from Shwepyitha Township were reared for mass production. The method of mass rearing of elephant mosquito larvae in laboratory was done according to Toma & Miyagi [9], but some improvement was done in this method. For mass rearing, 50-100 *Toxorhynchites splendens* larvae were reared individually in 50 ml beakers in the insectarium at 25-26°C, with 80% relative humidity (RH) and 16hr

light and 8hr dark photo-period. During their first and second instars of *Toxorhynchites splendens* larva, laboratory reared 1st and 2nd stages of *Ae. aegypti* larvae were fed, then 3rd and 4th *Ae. aegypti* larvae were fed during later instars of *Toxorhynchites* (fifty each 3rd and 4th stage prey larvae were provided daily to each *Toxorhynchites* larva).

The adult *Toxorhynchites splendens* mosquitoes were housed in large cage (3ftx2ftx 2ft) covered with cotton mosquito net and were given 10% glucose with burplex as food. The glucose supplement was provided throughout their adult life and maintained oviposition in the cage for next generation.

Laboratory study

Initial laboratory trials were conducted at Medical Entomology Section, National Health Laboratory (NHL). In the laboratory, the longevity of elephant mosquito's larval life, maximum number of larval consuming rate and favourable food using *Aedes aegypti*, *Culex* larvae and *Tubifex* spp. as larval food were determined. Larvivorius tests were carried out according to WHO instruction [10].

Field study

Before intervention, *Aedes* larvae positivity in all kinds of water storage containers (major, minor and miscellaneous) was detected and recorded in both test and control areas. During intervention, second stage two *Toxorhynchites* larvae each were put into all kinds of water storage containers every two weeks and larval positivity was recorded monthly for 3 months (before, during and after intervention month). Before and after intervention, House Index (HI), Container Index (CI), Breteau Index (BI) and percentage reduction of key containers (>500 larvae/container) and positive premises (3 & above positive containers/ house) were calculated.

Field trial

Intervention area

Fifty houses were randomly selected to

introduce elephant mosquito larvae in domestic water containers. Laboratory reared two second-instars *Toxorhynchites* larvae were treated every fourteenth day in each larval negative major containers (metal drum, bago jar, concrete tanks, earthen pot and jars etc.) and at the same time one *Toxorhynchites* larva each was treated in minor (small earthen jar, alter flower vases, spiritual bowl, ant trap etc.) and miscellaneous (discarded tire, batteries, small earthen pot, tin can etc.) containers. Two to three *Toxorhynchites* larvae were introduced to positive containers according to the larval density.

Non-intervention area

50 houses were randomly selected for *Aedes* larvae positivity and compared with the results of intervention area. Surveys were undertaken in both areas prior to intervention and monthly post-intervention surveys were conducted for three months. *Aedes* larval positivity in pre and post-intervention surveys was measured in all domestic water containers (major, minor and miscellaneous) and recorded.

Measurement of outcome indicators

House Index (HI)	= Percentage of houses positive for <i>Ae. aegypti</i> larvae
Container Index (CI)	= Percentage of water holding containers infested with <i>Ae. aegypti</i> larvae
Breteau Index (BI)	= Number of containers with <i>Ae. aegypti</i> breeding per 100 houses searched
Key container	= Those containers with >500 larvae
Positive premises	= Households with 3 and above positive water containers for <i>Ae. aegypti</i> larvae

Analysis of data

All laboratory and field data were analyzed by using Microsoft EXCEL. Larval indices and percent reduction of key containers and key premises and Chi square test and p value were calculated.

RESULTS

Seasonal distribution

Elephant mosquito larvae were found monthly in fields but highest in August followed by July and September. Maximum numbers of elephant larvae were collected in old car tires and old bago jars followed by earthen pots and black plastic pots in field areas. The species of adult elephant mosquito was identified as *Toxorhynchites splendens*.

Mass rearing result

Individual *Toxorhynchites splendens* female laid 20-40 eggs in black plastic bowl and bamboo cup in laboratory. They laid 15-18 times in their life span. The adult female life span was 60-70 days.

Laboratory results

The longevity of the elephant mosquitoes larval life were found as 21 ± 3 , 24.5 ± 4 and 28.3 ± 4 days when fed with *Aedes* larvae, *Culex* larvae and *Tubifex* spp, respectively. They preferred *Aedes* to *Culex* larvae (45.3 vs. 35.4) per day as food for mass production of elephant mosquito larvae in laboratory.

Fig. 1 shows that single 4th stage elephant mosquito larvae consumed the highest number (129 ± 8) of 1st and 2nd and 45.3 ± 5 number of third and fourth stages of *Aedes* larvae/day. Single 2nd stage elephant mosquito larvae showed higher killing behavior i.e. 23.6 ± 5 *Aedes* larvae/day killed in 200 larvae/1000 ml water. For effectiveness, 2nd stage elephant mosquito larvae were selected for controlling DHF vector *Aedes* in the field.

Table 1. Monthly variation of indices before, during and after intervention period in intervention and non-intervention areas.

Study areas	Before intervention			During intervention						After intervention		
	(May)			(June)			(July)			(August)		
	HI %	CI%	BI	HI%	CI%	BI	HI%	CI%	BI	HI%	CI%	BI
Intervention test area (Shwepyitha Township)	92	36.4	356	56	13.55	132	32	5.95	58	4***	0.6***	6***
Non-intervention control area (North Dagon Township)	90	38.2	298	86	37.21	288	86	36.95	286	80	35	277

HI= House Index, CI= Container Index, BI = Breteau Index

(HI $\chi^2=52.24$, $p=0.001***$; CI $\chi^2=22.83$, $p=0.021***$; BI $\chi^2=228.02$, $p=0.001***$)

Field results

Table 1 shows that after the intervention, House Index (HI), Container Index (CI) and Breteau Index (BI) were significantly reduced in intervention area (Shwepyitha) compared to non-intervention area (North Dagon). HI, CI and BI were reduced from 92%, 36.40% and 356 to 4% ($\chi^2=52.24$; $p=0.001***$), 0.62% ($\chi^2=22.83$; $p=0.021***$) and 6% ($\chi^2=228.02$; $p=0.001***$) within 3 months in Shwepyitha but in non-intervention area, the larval indices were not significantly reduced.

Table 2 shows that 93.75% reduction was evident in number of key containers (>500 larvae positive/ container) ($\chi^2=7.88$; $p=0.049*$). Table 3 shows that 95.24% reduction was evident in positive premises

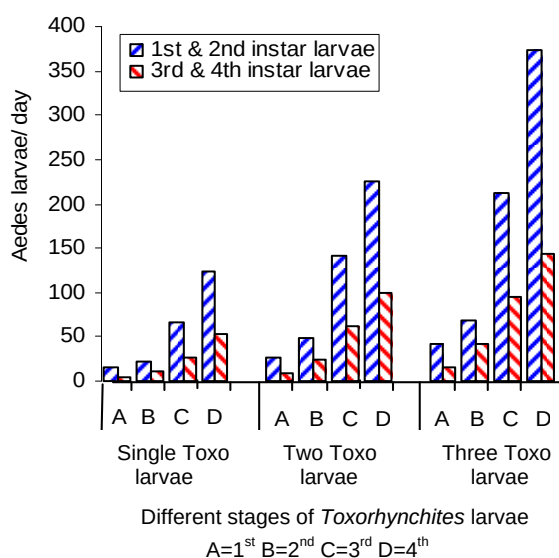


Fig.1. Predatory rates of 1st to 4th instar elephant mosquito larvae/day on *Aedes* 1st and 2nd instars and 3rd and 4th instar larvae in 1000 ml tap water

(3 & above containers positive with larvae/house) ($\chi^2=9.71$; $p=0.001^*$) in intervention area of Shwepyitha Township. Highest number of *Aedes* larvae breeding containers was found in metal drums, concrete jars, bago jars, candle making machines, large flower vases, spiritual bowls and discarded car tires. The breeding grounds of *Aedes* were found in old car tire shops, concrete jar making industries, earthen pot shops, candle making industries and old refrigerator repairing shops.

Table 2. Comparison of percentage reduction of key containers (before and after intervention) in intervention and non-intervention control areas

Study areas	Before intervention	After intervention	Percentage reduction (%)
Intervention area (Myoma Ward, Shwepyitha Township)	16	1	93.75
Non-intervention control area (Ward 31, North Dagon Township)	17	14	17.65

Key container =>500 mosquito larvae positive/containers ($\chi^2=7.88$, $p=0.049^*$)

Table 3. Comparison of percentage reduction of positive premises (before and after intervention) in intervention and non-intervention control areas

Study areas	Before intervention	After intervention	Percentage reduction
Intervention area (Myoma Ward Shwepyitha Township)	21	1	95.24
Non-intervention control area (Ward 31, North Dagon Township)	19	17	10.526

Positive premises =>3& above containers positive for mosquito larvae/household ($\chi^2=9.71$, $p=0.001^*$)

DISCUSSION

The elephant mosquito larvae are predatory on other mosquitoes larvae and show "prepupal killing behaviors" (before pupation they kill) and in addition, female *Toxorhynchites* spp. oviposit into pools of water which are not accessible to chemical control methods [11]. The 2nd stage elephant

mosquito larvae showed highest killing behavior than 3rd and 4th stage elephant mosquito larvae. The longevity of larval life is 21-22 days which is enough for destroying *Aedes* larvae in field for every 14-day trial. A laboratory study of Indian researcher in Pondicherry India revealed that in the case of second instar predators, prey size selection was not dependent on second instar to fourth instar prey [12].

Field studies showed that elephant mosquito larvae were highly effective in control of *Aedes* mosquito immature stages. Reduction in *Aedes* larvae indices after intervention was a valuable finding. Key containers and positive premises were also significantly reduced from pre- to post-intervention surveys in test area ($\chi^2=7.88$, $p=0.049$ and $\chi^2=9.71$, $p=0.001$). It was comparable to other biological agents such as larvivorous fish and dragonfly nymphs. One study revealed that *Aedes aegypti* larvae were effectively controlled by larvivorous fish in metal drums [13]. Dragonfly nymphs were also very effective biocontrol agents of container breeders of *Aedes aegypti* [14]. A biocontrol study of *Aedes* larvae using dragonfly nymphs in Thakayta Township observed that larval indices were significantly reduced (over 90% reduction in key containers and key premises) from before intervention month to after intervention month [15].

Second-instar *Toxorhynchites splendens* larvae were also used successfully to suppress *Ae. aegypti* and *Ae. albopictus* in domestic water containers in Malaysia [6], Fourth-instar *Toxorhynchites splendens* larvae were used successfully to reduce the *Ae. aegypti* in Sa-med Island, Thailand in 1979 [16] and in Pudapet, a coastal village of India [17]. Also *Toxorhynchites amboinensis* (as *Toxorhynchites splendens*) were introduced into Manila in 1953 [18]. American Samoa in 1978 effectively controlled the filariasis vector *Aedes polynesiensis* Marks [19, 20] and *Cu. Quinquifasciatus* (say 1829) in Darjeeling India by *Toxorhynchites splendens* larvae [21].

A study in Java mentioned that *Toxorhynchites splendens* larvae had been successfully controlling *Aedes* larvae population but less successful in domestic water storage containers due to the large number of untreated natural containers in which target mosquitoes were breeding [22].

Elephant mosquito larvae were easily found in unused bago jars, earthen pots, and discarded old car tyres. Mostly they were found together with *Aedes* larvae due to the fact that *Aedes* larvae were favorite food prey for them. Highest number of *Aedes* larvae breeding was found in metal drums, concrete jars, bago jars, candle making machines, large flower vases, spiritual bowls and discarded car tyres. The breeding grounds of *Aedes* were found in old car tyre shops, concrete jar making industries, earthen pot shops, candle making industries and old refrigerator repairing shops. These are the targeted containers and targeted places for future study. This control method is locally available and harmless to man and environment. The elephant mosquito larvae could be collected easily in long-term unused or discarded water storage containers. It is feasible for controlling water storage container breeder of DHF vector *Aedes aegypti* mosquito's larvae in all kinds of water containers.

Conclusion

Biological method of elephant mosquito larvae was found to be effective in suppression of *Aedes* mosquito's population in control of DHF in the community. The method was regarded as acceptable and feasible in study household. This control tool is locally available and harmless to man and environment. Therefore, application of elephant mosquito larvae should be considered as an alternative in control of DHF when routine methods fail. The biological control method removed both larvae and pupae of *Aedes aegypti* simultaneously. The elephant mosquito larvae could be collected in long-term unused or discarded water storage containers. *Toxorhynchites* spp. adult does not blood feed but they feed glucose

from flowers and therefore cannot themselves act as vectors of disease and cannot be involved in transmission of diseases.

Applicability and recommendations

In urban and semi-urban areas where *Aedes aegypti* is the main vector of DHF and the breeding sites are permanent, elephant mosquito larvae could be used in addition to existing control measures. The method is very appropriate for grass-root level where DHF prevailed most. The *Toxorhynchites* spp. could be collected in long-term unused water containers and discarded water storage containers. Application of elephant mosquito larvae for controlling *Aedes aegypti* in the community should be encouraged. Control should be formed under the existing National Mosquito Control Committee (NMCC) to exert more concerted efforts in DHF control activities using cost-effective methods and should include information on vector bionomics, key containers, risk factors and risk behaviour.

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Evaluation of antidiarrhoeal activity of *Swertia angustifolia* Ham. (ဝနံးခါး) on mice

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In order to combat the problems of diarrhoea globally, the World Health Organization encouraged studies for the treatment of diarrhoea diseases with herbs. It thus becomes important to evaluate commonly available natural drugs as alternative to currently used antidiarrhoeal drugs. The study was carried out to evaluate acute toxicity, phytochemical, and antidiarrhoeal activity of *Swertia angustifolia*. Acute toxicity study showed that there was no toxic effect even with maximum permissible dose of 24 g/kg. No toxic compound and metal like cyanogenic glycoside and arsenic were detected. *In vivo* antidiarrhoeal activities of aqueous extracts (3, 6, 12 g/kg single oral doses) were carried out using castor oil-induced diarrhoea model in mice with standard loperamide. Like loperamide, all doses of extract produced significant reduction in fecal output and prolongation in onset of diarrhoea ($p < 0.05-0.0005$) compared with that of control. To understand the mechanism of action, it is also evaluated castor oil-induced gastrointestinal motility (intestinal-transit) and intestinal fluid accumulation. The extract and loperamide also significantly reduced the castor oil-induced gastrointestinal motility and intestinal fluid accumulation (anti-secretory effect) ($p < 0.05-0.0005$). The *in vivo* antidiarrhoeal index of extract and loperamide were 60.2% and 97.4%, respectively. Antibacterial activity screening of aqueous extracts against 9 types of pathogenic microorganisms was carried out by employing agar disc diffusion method. It inhibited the growth of enterotoxigenic *Escherichia coli* (ETEC), *Salmonella typhi* and *Salmonella paratyphi*. These results indicated their potential usefulness in infected diarrhoea with these three organisms. In conclusion, aqueous extract of *Swertia angustifolia* revealed the antidiarrhoeal effect and potential utility in both infected and non-infected diarrhoea states.

INTRODUCTION

Diarrhoea is an intestinal disorder characterized by abnormal fluidity and frequency of fecal evacuates generally the result of increased motility in the colon and the passage of loose or watery stools, usually at least three times in a 24-hour period [1]. In developing countries, diarrhoeal disease is one of the leading causes of childhood morbidity and mortality and more than 5-8 million deaths each year in infants and children especially in under five years of age [2]. In 2001, the World Health Organization (WHO) estimated 1.5 million

below 5 years died from diarrhea [3]. Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems and associated with excessive defecation and abnormally loose consistency [4]. In order to combat the problems of diarrhoea globally, WHO has given a special emphasis on the use of traditional medicines in the control and management of diarrhoea [5]. It thus becomes important to evaluate commonly available natural drugs as alternative to currently used antidiarrhoeal drugs which are not always free from adverse effects. In Myanmar, diarrhoea is one of the six major priority diseases, and

according to the priority ranking of diseases based on scoring system in National Health Plan, diarrhoea and dysentery ranked the 4th position. The 4th leading cause of morbidity rate due to diarrhoea and gastroenteritis of presumed infectious origin was reported 3.6% in 2008 [6].

Nine *Swertia* species including *Swertia angustifolia* occupied one of the major positions in trade of non-timber forest products in Nepal because of its uses to substitute *Swertia chirayita* which is a powerful/effective non-toxic cure for various diseases such as bronchial asthma, diabetes, fever, gastric ulcer, hepatitis, urinary disorder and malaria [7]. *Swertia angustifolia* Ham. (family Gentianaceae) is commonly grown and easily available in Myanmar and used to treat malaria, diabetes, fever, hepatitis, hypertension and diarrhoea. But there was no scientific report on antidiarrhoeal activity of *Swertia angustifolia* in Myanmar. Hence, this study was aimed to evaluate *in vivo* and *in vitro* antidiarrhoeal activity of reputed *Swertia angustifolia*, its mechanism of action and to screen the antibacterial activity of various extracts against 9 types of pathogenic bacteria.

MATERIALS AND METHOD

Preparation of extracts

The samples were collected from Demoso Township, Kayah State. They were identified by a taxonomist from Department of Botany, Yangon University as *Swertia angustifolia* Ham. Aqueous extracts were prepared according to the method of Geissman [8].

For quality control

Phytochemical and physicochemical tests were carried out on the dried whole plant powder sample using standard procedure as described by Harbone [9] and Central Council of Research in Unani Medicine [10]. Composition of the elements was studied by Energy Disperse Xray Fluorescence Spectroscopy.

Acute toxicity test

The acute toxicity test was done to determine the symptomatology consequence, degree of toxicity to administration of the drug and to find out the median lethal dose (LD₅₀) by using the method of Litchfield and Wilcoxon [11]. A total of 40 mice, ddy (Dutch Denken Yoken) strain, were randomly divided into 4 groups (I-IV) of 10 animals each. The mice of group I, served as control, were administered with 0.1 ml/10g of distilled water orally and group II to IV were treated orally with 6, 12, and 24 g/kg aqueous extract of *Swertia angustifolia* Ham. The mice were observed hourly for the first 12 hours and then daily for 2 weeks. Changes in appearance, behaviors, neurologic and autonomic signs were noted as described by Loomis [12].

Study on in vivo anti-diarrhoeal effect of Swertia angustifolia Ham.

These were studied by castor oil-induced diarrhoea, enteropooling and small intestinal transit test.

Castor oil-induced diarrhoea

A total of 30 mice were divided into 5 groups, each contained 6 mice. Group I, served as control, received normal saline 10 ml/kg body weight, group II received standard loperamide, 6 mg/kg body weight, and group III, IV and V were treated with 3, 6 and 12 g/kg body weight of aqueous extracts of *Swertia angustifolia* Ham. 1 hour before castor oil administration of 10 ml/kg body weight. The onset and number of diarrhoea droppings were counted hourly for 4 hours, mean onset and mean of the droppings passed by the treated groups were compared with that of the control group according to Awouter, *et al.* [13]. The percent inhibition of defecation was calculated as follows:

$$\% \text{ inhibition of defecation} = \frac{\text{Control} - \text{Test} (\text{Mean defecation})}{\text{Control mean defecation}} \times 100$$

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al.*, [14]. Overnight fasted mice were divided into 3 groups of six animals each. Group I, served as a control, received normal saline (10 ml/kg) orally, group II received loperamide (6 mg/kg) and group III received aqueous extract of *Swertia angustifolia* Ham. (6 g/kg), 1 hour before castor oil administration of 10 ml/kg. One hour later, the mice were sacrificed, the small intestines were tied at both ends, removed and weighed. The intestinal contents were collected by milling into a beaker and their volumes were measured by measuring cylinder. The intestine was reweighed and the difference between full and empty intestine was calculated as the weight of faecal matter.

Castor oil-induced small intestinal transit

Mice were fasted for 18 hours and divided into three groups, each contained six animals. Group I received normal saline (10 ml/kg) orally, group II received loperamide (6 mg/kg), group III received aqueous extract of *Swertia angustifolia* Ham. (6 g/kg) orally, 1 hour before administration of castor oil. Maker (10% charcoal suspension in 5% gum acacia, 10 ml/kg) was administered orally 1 hour after oil treatment. After thirty minutes, the mice were sacrificed and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [15], percent inhibition of intestinal transit, antidiarrhoeal index (ADI) were calculated [16] as follows:

$$\% \text{ intestinal transit} = \frac{\text{Charcoal meal travelled}}{\text{Total length of intestine}} \times 100$$

$$\% \text{ inhibition} = \frac{\text{Total length} - \text{Charcoal meal travelled}}{\text{Total length}} \times 100$$

$$\text{ADI}_{in\ vivo} = \sqrt[3]{D_{freq} \times G_{mag} \times P_{freq}}$$

D_{freq} = delaying defecation time of diarrhoea onset, in percent of control

G_{mag} = gut-meal travel distance reduction, in percent of control.

P_{freq} = purging frequency as number of stool reduction, in percent of control

$\text{ADI}_{in\ vivo}$ = *In vivo* antidiarrhoeal index, in percent

In vitro screening of antibacterial activity

The antibacterial activities of the aqueous extracts against 9 species of pathogenic *enterotoxigenic Escherichia coli* (ETEC), enteropathogenic *Escherichia coli* (EPEC), and enteroaggregative *Escherichia coli* (EAEC), *Salmonella typhi*, *Salmonella paratyphi*, *Vibrio cholerae*, *Shigella dysenteriae*, *Shigella boydii*, and *Shigella flexneri* were carried out by employing agar disc diffusion method [17].

RESULTS

The morphology, taxonomy of the plants was observed to agree with *Swertia angustifolia* Ham. [18], documented at Botany Department, Yangon University (Fig. 1).

Preparation of extracts

The aqueous extraction of 100 g of dried crude powder yielded 14.9 g of dried extract (14.9%).

Quality control test

The dried crude powder of *Swertia angustifolia* showed the presence of glycosides, flavonoids, steroids/ triterpene, saponins, and reducing sugar. However, alkaloids, tannins, amino acid and cyanogenic glycosides were absent. Potassium and calcium were the major elements and iron, zinc, manganese, and chloride were found as trace elements. Physicochemical properties showed moisture content 6.0%, total ash content 6.7%, water soluble ash content 12%, water soluble matter 14.9%,

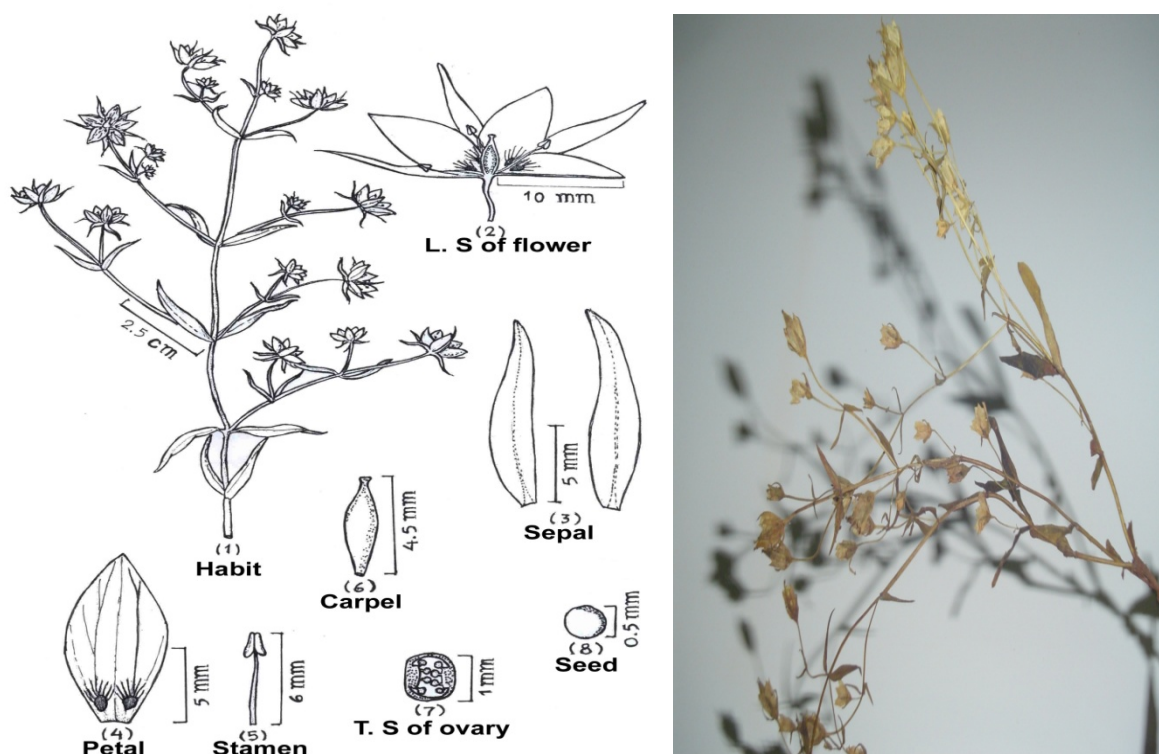


Fig. 1. Diagnostic characters of *Swertia angustifolia* Ham.

methanol soluble matter 14.5%, ethanol 7.6%, acid soluble ash content soluble matter 15.3%, ethyl acetate soluble matter 5.7% and pet ether soluble matter 8.4% .

Acute toxicity test

The acute toxicity test was done to find out the median lethal dose (LD₅₀) by using the method of Litchfield and Wilcoxon [11]. Acute toxicity study showed that there was no toxic effect even with maximum permissible dose of 24 g/kg. Therefore, the LD₅₀ of *Swertia angustifolia* Ham. was supposed to be more than 24 g/kg body weight.

In vivo antidiarrhoeal effects

Castor oil-induced diarrhoea

After thirty-minute administration of castor oil, the typical diarrhoea signs such as watery and frequent defecation were shown in all animals of control group up to 4 hours. The onset of diarrhoea was found to be significantly delayed by all aqueous extracts as well as loperamide (6 mg/kg) ($p < 0.05$ - $p < 0.001$) when compared with control

and the mean frequency of defecation over four hours was found to be significantly decreased by all aqueous extracts as well as loperamide (6 mg/kg) ($p < 0.05$ - $p < 0.001$) when compared with control (Table 1).

Table 1. Antidiarrhoeal effects of aqueous extracts and standard drug loperamide on castor oil-induced diarrhoea in mice

Treatment	Onset of diarrhoea (mins)	Mean defecation in 4hr	% Inhibition of defecation within 4hr
Control (castor oil only)	26.67±4.2	18.83±2.44	
Castor oil+ aqueous extract (3 g/kg bw)	51.5±8.8*	8.5±1.09*	54.86726
Castor oil+ aqueous extract (6 g/kg bw)	89.17±23.8*	6.83±2.36**	63.71681
Castor oil+ aqueous extract (12 g/kg bw)	83.5±12.3***	8.0±0.52***	57.52212
Castor oil+ loperamide (6 mg/kg bw)	148.33±15.15***	6.0±2.02****	68.14159

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$
bw= body weight

The peak activity was found at 6 g/kg dose of test extract which is similar to that of loperamide. The percent inhibitions of defecation over four hours of aqueous extracts (3, 6, 12 g/kg) and loperamide (6 mg/kg) were 54.87%, 63.72%, 57.52% and 68.14%, respectively.

Castor oil-induced enteropooling

The antidiarrhoeal effects were examined by the decreasing in weights as well as the volumes of the intestinal fluid contents induced by castor oil. The experiment was conducted using loperamide (6 mg/kg) and aqueous extracts (6 g/kg). The aqueous extract significantly decreased the intestinal content in weight (32.16%; $p<0.05$) and in volume (35.99%; $p<0.01$) when compared with control. Therefore, the aqueous extracts had less anti-enteropooling activity than standard loperamide which showed reductions in weight and volume of intestinal content as 58.97% and 59.81%, respectively (Table 2a).

Table 2a. Comparative antidiarrhoeal efficacies of aqueous extract and loperamide on castor oil-induced enteropooling and intestinal transit in mice

Treatment	Enteropooling				Intestinal transit	
	Intestinal content				%	%
	W (g)	% Rw (g)	V (ml)	%Rv (ml)	Intestinal transit	Inhibition
Control	1.03		0.98		60.73	39.27
(castor oil only)	±		±		±	±
	0.17		0.04		5.04	5.04
Castor oil+ Loperamide (6mg/kg bw)	0.38	58.97	0.43	59.81	37.29	62.71
	±	±	±	±	±	±
	0.07**	13.45	0.06 [#]	9.98	2.31 ^ψ	2.31 ^ψ
Aqueous extract (6g/kg bw)	0.54	32.16	0.61	35.99	48.13	51.87
	±	±	±	±	±	±
	0.12*	22.01	0.09**	10.11	2.28*	2.28*

* $p<0.05$, ** $p<0.01$, ^ψ $p<0.005$, [#] $p<0.0005$

W=Weight Rw=Reduction in weight V=Volume Rv=Reduction in volume

Castor oil-induced small intestinal transit

The percent intestinal transits were significantly reduced from 60.73 ± 5.04 to 48.13 ± 2.28 and 37.29 ± 2.31 ($p<0.05$ - 0.005), respectively, after treating with aqueous extract (6 g/kg) and loperamide (6 mg/kg) were 51.87% and 62.71%, respectively.

Therefore, the percent inhibition of aqueous extract was also found to be comparable with standard loperamide (Table 2a). These indicated that the aqueous extracts and loperamide significantly reduced the weights and volumes of intestinal fluid secretions as well as intestinal transit produced by castor oil. This showed that aqueous extract and loperamide had anti-secretory and anti-motility effect.

In vivo antidiarrhoeal index in percent for aqueous extracts (6 g/kg) and standard drug loperamide (6 mg/kg) were calculated and found to be 60.28% and 97.46%, respectively (Table 2b).

Table 2b. Comparative antidiarrhoeal efficacies of aqueous extract and loperamide on castor oil-induced *in vivo* mice model

Treatment	Delaying defaecation time of onset in mice (min)	Gut meal travel distance in mice (cm)	Purging frequency in mice (number of stool)	<i>In vivo</i> anti-diarrhoeal index (%)
Control	35.83	28.5	18.83	
(castor oil only)	±	±	±	0.00
	5.07	2.68	2.44	
Loperamide (6 mg/kg)	148.33	16.17	6	
	±	±	±	97.46
	15.14 [@]	1.08 ^ψ	1.31 [#]	
Aqueous extract (6 g/kg)	89.17	21.92	6.83	
	±	±	±	60.28
	23.78*	1.11*	2.35**	

* $p<0.05$, ** $p<0.01$, ^ψ $p<0.005$, [#] $p<0.001$, [@] $p<0.0005$

Antibacterial activity

In this investigation, the samples were tested on 9 types of bacteria and inhibition zone diameters were recorded. Aqueous extracts inhibited the growth of *E. coli* (ETEC), *Salmonella typhi* and *Salmonella paratyphi* with inhibition zone diameters of 20, 21 and 20 mm, respectively.

DISCUSSION

Several drugs are being used in medical practice for the symptomatic relief of diarrhoea. Kaolin-pectin, bismuth-sub-salicylate, codeine phosphate and diphenoxylate with atropine (lomotil) have been available for many years. Loperamide

hydrochloride has been introduced more recently than others and is being reported superior to others [19]. The potential for abuse, the presence of undesirable side effects, and lack of gastro-intestinal specificity associated with certain currently available antidiarrhoeal agents, have impelled research efforts to discover new antidiarrhoeal agents which can act directly on the intestine and do not have significant side effects.

Diarrhoea results from the imbalance between the absorptive and secretory mechanisms in the intestinal tract, resulting in the excess loss of fluid in the faeces. In some diarrhoea, the secretory component predominates, while other diarrhoea are characterized by hypermotility. The use of castor oil-induced diarrhoea model in our study is logical because the autocoids and prostaglandins are involved these have been implicated in the causation of diarrhoea in man [20]. Castor oil is also reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins, which stimulate motility and secretion, thereby prevents the reabsorption of NaCl and water [21]. Probably the aqueous extract of *Swertia angustifolia* increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

The results of this study showed that the aqueous extract of *Swertia angustifolia* produced significant reduction in frequency of diarrhoea induced by castor oil and produced significant delayed onset of diarrhoea. It is also noted that the extract showed significant inhibition on castor oil-induced intestinal fluid accumulation and reduction in the volume of intestinal content as well as intestinal transit.

The antidiarrhoeal activity of the extract may also be due to the presence of denature

proteins forming protein tannates. Protein tannates make the intestinal mucosa more resistant and reduce secretions. The secretory diarrhoea is associated with the activation of Cl-channels, causing Cl-efflux from the cell, the efflux of Cl-results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea [22]. Thus, the aqueous extract of *Swertia angustifolia* may inhibit the secretion of water into the lumen by reverting this mechanism.

Phytochemical screening revealed the presence of glycosides, flavonoids, steroids/triterpene, saponins, and reducing sugar in its dried powder. Antidysenteric and anti-diarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars [21]. Hence, reducing sugars, terpenoid and/or flavonoids may be responsible for the mechanism of action of its antidiarrhoeal activity.

Aqueous extract of *Swertia angustifolia* (6 g/kg) had significantly lower anti-diarrhoeal index (*in vivo*) in percent when compared with that of standard loperamide (6 mg/kg) in this study. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility. Reduction in gastrointestinal motility is one of the mechanisms by which many antidiarrhoeal agents act [23]. However, inhibited castor oil-induced enteropooling, thereby, suggesting that mediators other than cetylcholine are involved in castor oil-induced enteropooling [23].

For the *in vitro* anti-bacterial activity examination of crude extracts, agar disc diffusion method was used because of its simplicity, speed of performance, economy and reproducibility [24]. In some cases, there has been found that antidiarrhoeal activity is associated with the antimicrobial activity. In present study, the aqueous extract of *Swertia angustifolia* exhibited significant antimicrobial activity against

ETEC, *Salmonella typhi* and *Salmonella paratyphi*. These pathogens cause a variety of diseases including diarrhoea and gastroenteritis in human [25]. This result indicated their potential usefulness in infected diarrhoea with these three organisms.

These data clearly showed that the aqueous extract of *Swertia angustifolia* revealed antimicrobial activity on three organisms and antidiarrhoeal activity due to anti-secretory and antomotility action. In conclusion, the extract showed potential utility in a wide range of both infected and noninfected diarrhoea states. It may be useful in disorder of transit eg. functional diarrhoea, radiation diarrhoea and disorder of abnormal secretory mechanisms like in *E. coli* enterotoxin-induced diarrhoea.

It is recommended for isolation and structural elucidation of active antidiarrhoeal agents from *Swertia angustifolia* Ham. and to determine the acute, subacute toxicity study for safety of *Swertia angustifolia* Ham to introduce clinical trial on human being.

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Community acceptance on insecticide treated bed-nets in selected rural communities

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Malaria control program in collaboration with Non-Governmental Organizations has been launched Insecticide Treated Bed-nets (ITNs) projects in selected malaria endemic areas since 2002. This study investigated the community perception, acceptance and utilization of ITN in the selected rural communities in PyinOoLwin Township. Four focus group discussions were carried out in March, 2010. These discussions explored community perceptions, acceptability, preference on types of bed-net and the barriers to use ITNs. Free availability, mosquito nuisance, and popularity were the main determinants of ITN use. Forest related works and evening social activities had negative impact on compliance with ITN usage. Majority of participants in Twin-nge Village (received free ITN for every household) reported using ITN regardless of any unfavorable characteristics. Most of the participants in Si-kar Village (received limited ITN distribution) described unfavorable characteristics of ITN as reasons for their intermittent use or non-compliance. The main reason for ITN acceptability was effectiveness in killing mosquitoes and other insects. Discussions highlighted considerable confusion around ITN care and washing. Not only adequate and free distribution based on sex and age composition of household members, but also provision of information about ITNs will be important for improving compliance.

INTRODUCTION

Malaria is still remaining as one of the leading causes of morbidity and mortality and a disease with public health implications in Myanmar. The humanitarian dimension of malaria in Myanmar is enormous as a large proportion of the population is affected. Out of estimated total population of 54.28 million (2004), 38.54 million (71%) live in malaria endemic areas. The disease is endemic in 284 out of 324 townships, mainly in rural areas and in some suburban areas [1].

The morbidity and mortality rates of malaria have dropped from 24.3/1,000 and 11.1/100,000 population, respectively, in 1990 to 12.6/1,000 and 3.7/100,000 population, respectively, in 2004 [2]. The major approaches to reduce the malaria morbidity and mortality in Myanmar include:

increasing accessibility to quality diagnosis and appropriate treatment according to national anti-malaria treatment guideline and scaling up the ITN program throughout the country. With limited resources, areas were prioritized for either distribution of Long Lasting Insecticidal Nets (LLIN) or impregnation of existing nets. In 2008, 71,605 LLINs were distributed in 824 villages of 17 endemic townships, particularly in hard-to-reach areas while a total of 354,079 nets were impregnated in 2982 villages of 50 endemic townships. Total population covered by ITN program was 3,147,719 [3].

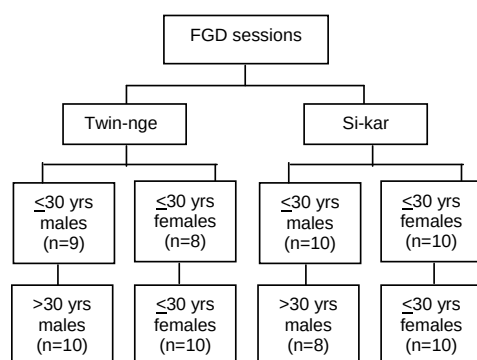
Despite this demonstrated efficacy of ITN program, the choice, use, and acceptance of ITNs continue to face major socio-economic and cultural challenges in most malaria endemic areas [4, 5]. Compliance with ITN usage is dependent on their acceptability in

the socio-cultural context of the local population. This study investigated the community perception, acceptance and utilization of ITN in selected rural communities of PyinOoLwin Township where ITN programs are currently running.

MATERIALS AND METHODS

A community-based cross-sectional descriptive study was carried out with a qualitative approach using focus group discussions (FGDs) with local people in March, 2010. The study communities were purposefully selected to capture the views of those living in areas where ITN programs are currently running. Two villages were selected based on availability of ITNs; Twin-nge Village (every household received ITN free-of-charge), and Si-kar Village (there was limited ITN distribution in the village).

A total of 75 people, 37 people from Twin-nge and 38 people from Si-kar Village, participated in this study. A total of eight FGD sessions, two FGD sessions with younger males and females (≤ 30 years) and two FGD sessions with older males and females (> 30 years) in each village, were conducted with eight to ten participants in each session. They were selected by village administrative bodies with criteria of inclusion in specified age groups, willingness to participate in the study, permanent residence in the study village, being the head of household or housewife who received ITN, and who can speak Myanmar language.



All FGD sessions were facilitated by principal investigator. Note taking was performed by trained research officers and research assistants. A structured interview guide was used to conduct FGD sessions, which focused on knowledge of the community about malaria, availability and benefits of ITNs, socio-cultural barriers to utilization of ITNs, ITNs using practices, the acceptance of the communities on ITNs, and suggestions on ITN program. All FGD sessions were video recorded with consents of participants. The transcripts were written in Myanmar using Win-innwa font in Microsoft Word version 2007. Data were coded around the main topics of the interview guide and entered into Atlas Ti version 6.02-software. Main themes were drawn out of the interviews and subjected to thematic analysis by principal investigator and co-investigators. Areas of consensus and divergence were identified in the analysis of this study.

Ethical consideration

Ethical approval was obtained from Institutional Ethical Committee of Department of Medical Research (Upper Myanmar). Written informed consent was obtained from each and every respondent.

RESULTS

Background characteristics of participants

Eight FGD sessions were carried out with 75 local people. Among them, 37 people were males and they were household heads and the remaining 38 people were housewives. The age of the participants ranged from 24 to 29 for younger male FGD session, 32 to 64 for older male FGD session, 22 to 28 for younger female FGD session, and 39 to 54 for older female FGD session. Majority of people were Danu ethnic and it was followed by Shan and Bamar. All of the participants can read and write, and majority of the participants passed primary school. A background noise in Twin-nge Village was inclusion of a member of an INGO in older male session.

It was identified during discussion. The facilitator requested him to talk after the other peoples' conversation. The facilitator encouraged him that "you know much. So, let the other people talk first. Then you may add necessary information." However, he was so talkative that the facilitator managed this problem by assigning a research assistant to do an in-depth interview with this participant at the next house. With due regards to research methodology, information obtained from that respondent was not analyzed in the study.

Knowledge on mode of transmission of malaria

Male participants reported that malaria is caused by drinking stream water in the forest. This statement was repeated for six times during male FGD sessions and no one in the group rejected that statement as a misconception. They specified that drinking "phet-sut-yae" (ဖုတ်ဆုတ်ရေ) in local language might cause malaria. According to their words, phet-sut-yae is water from a pool in the jungle, in which decayed leaves sank and mosquitoes lay off eggs in it. If somebody drinks it in the forest, they might get malaria. Some misconceptions regarding the cause of malaria included going into the malaria endemic forest (ခုန်တော), traveling under the rain and association with common cold.

"We have to drink phet-sut-yae in the forest. It is not clean. There are many mosquitoes in the forest and these mosquitoes lay off eggs in the water. When we were thirsty, we drank it and got malaria."

(A 43-year-old male, Twin-nge Village)

On the other hand, some younger women revealed that malaria was caused by mosquito bite in the forest.

"Malaria mosquitoes stay in the forest. They don't stay in the village. People get malaria because they go into the forest. If they don't go into the forest, they wouldn't get malaria."

(A 27-year-old female, Twin-nge Village)

Knowledge on symptoms of malaria

Almost all participants reported that malaria is characterized by fever with chills and rigors accompanied by aches and pain, and no difference was observed in description on symptoms of malaria between male and female FGD sessions in both villages. According to their words, fever occurred at an exact time. Majority of malaria sufferers reported that fever was accompanied by aches and pain, back pain, chills, rigors, alopecia and fever usually subsided spontaneously. When fever subsided, the patient could take his meal and fever did not appear again on that day. Most of the sufferers reported that fever occurred every-day during illness while few said it appeared on alternate day. The sufferers of alternate day attack reported that the duration of fever was so long that some people used the word

"You are suffering from fever because you are lazy (အပျင်းများကြီးပါကွာ)."

(A 64-year-old male, Twin-nge Village)

"When I got malaria attack, I was shivering like riding a motor cycle."

(A 45-year-old male, Twin-nge Village)

"...shivering is so strong that the whole house was trembling..."

(A 41-year-old male, Si-kar Village)

Knowledge on treatment of malaria

Most of the younger people reported that they received curative treatment from midwives. According to their words, they received some injections and a variety of colorful tablets and none of them knew which one was antimalarial drug. Some elderly male participants were still relying on traditional remedies to treat malaria.

Perceived purpose of bed-nets use

Popularity, free availability, and protection from a variety of insects and mosquitoes bites were identified by all discussion groups as the main purpose of using bed-nets.

“Nowadays, bed-net usage becomes more popular, I think, there is no one who doesn’t want to use a bed-net.”

(A 47-year-old female, Si-kar Village)

“We did not use bed-nets before because we couldn’t afford to buy a bed-net. Now we can get it free-of-charge.”

(A 41-year-old female, Twin-nge Village)

“Usually, there were mosquitoes in the room when it became darker (late evening). Since we used the bed-net, there were no more mosquito noises.”

(A 22-year-old female, Si-kar Village)

“My children always had head lice. The lice had been seen until last year. Now, they sleep under ITN and have no lice.”

(A 30-year-old female at Si-kar Village)

“The head lice, mosquitoes, bed-bugs, cockroaches were all died. It is good for health. We were free from these insects’ bite.”

(A 64-year-old male at Twin-nge Village)

ITN utilization

A divergence of ideas in priority to sleep under bed-net and seasonal variations was observed among the participants. Most of the male participants would like to give priority to wife and children to sleep under bed-net at home whereas some female participants used to give ITN to her husband to bring into the forest.

“I allow my children and their mother (wife) to sleep under a bed-net to prevent them from mosquito bite. It is not necessary for me because I live with mosquitoes for lifetime.”

(A 47-year-old male, Si-kar Village)

“I usually give the bed-net to my husband because there are more mosquitoes in the forest.”

(A 29-year-old female, Si-kar Village)

“I used to sleep under the bed-net during rainy and cold seasons. In hot season, however, I do not use the bet-net.”

(A 26-year-old male, Twin-nge Village)

“I always sleep under bed-net in all three-seasons (ရွှေရိုးဆောင်းခြင်ထောင်ထောင်အိပ်ပါတယ်) because I feel safe under it.”

(A 22-year-old female, Twin-nge Village)

Participants reported that forest related works and evening social activities had negative impact on compliance with ITN.

“We usually sleep under the bed-nets at home. However, when we go into the forest for collecting the young bamboo shoots, mushrooms, hunting, growing or collecting crops, all family members cannot use bed-nets because we don’t have enough bed-nets. Bed-nets were left at home and the people who go into the forest usually carried only blankets.”

(A 41-year-old male, Twin-nge Village)

“Our family members usually sleep under the bet-nets. However, in some special occasions such as donation ceremony, wedding, anniversary of our village temple (ဘုရားပွဲ), we have to work starting from evening up to midnight at the monastery or at the home of donor. In those nights, we don’t go back home and we usually sleep together at the site of occasion without bed-nets.”

(A 35-year-old female, Si-kar Village)

Perception on distribution of ITNs

In Twin-nge Village, most of participants reported that although ITNs were distributed to all households in village, they don’t have enough bed-nets for the whole family.

“Every house received one bed-net because our village located in their inventory. However, there was an imbalance between numbers of bed-net supply and household members. One bed-net was sufficient for a family with three household members, but it became insufficient for a larger family with more than three household members.”

(A 43-year-old male, with 5 children)

“... at least two bet-nets are needed in my family, one for parents and another for children”.

(A 44-year-old female, with 4 children)

In Si-kar Village, participants reported insufficient distribution of ITNs. The NGO supplied bed-nets only for low income families and families with many children.

“Although there were a total of about 180 households in village, they gave only 50 bed-nets. Bed-nets were given to families with many children.”

(A 27-year-old female from Si-kar Village)

Misconceptions on ITNs

Participants reported that minimal information was provided at the point of distribution of ITNs. Some participants perceived that the protective effects of ITNs on mosquitoes were due to its smell and heat.

Participants reported the problems in using bed-nets as well as the adverse effects of ITNs.

“My mother is over 80 years old now. She faced problems when she slept under the bed-net. After waking up, she lost orientation whether she was facing either east or west. Moreover, when she slept under it, she became breathless. She was afraid of sleeping under ITN.”

(A 51-year-old female, Si-kar Village)

They also mentioned that side effects of ITNs were mostly in recently impregnated bed-nets and pre-impregnated bed-nets were free from harm.

“ITN caused nothing to me. I think I got a pre-impregnated bed-net from NGO. I mean, the bed-nets that I got was impregnated already. It was not impregnated just before giving it to me.”

(A 22-year-old female, Twin-nge Village)

However, most of the participants reported that ITN had no adverse effect.

“We experienced no problem with ITN. It is good”.

(Most participants, <30 years, FGD, Si-kar Village)

Maintenance of bed-nets

Participants described considerable varia-

tion in the frequency for washing ITNs. All of the male participants neglected the frequency of washing. Female participants reported the frequency of washing bed-nets varied from once a month, once every two months up to once every six months. Only a few participants were aware of the recommendations on how often their nets should be washed. Some participants also believed that the insecticide goes out with washing and they were waiting for the return of the malaria team to retreat their nets.

“They told us to wash impregnated bed-net every three months and to dry it under shadow.”

(A 22-year-old female, Si-kar Village)

“They told us that we could wash impregnated bed-net every three months. However, we usually wash once a month or once every two months when we feel that it was dirty.”

(A 27-year-old female, Si-kar Village)

Preference on types of ITN

Most of participants preferred large and strong bed-nets because most of them have large family members.

“The bed-net they gave was small. It was not appropriate for a family with many household members. It was not safe for children from mosquito bites. When I complained about it, they gave us a bigger one.”

(A 41-year-old male at Twin-nge Village)

Moreover, they preferred to get a suitable bed-net to use in the forest. Some of the participants suggested impregnating the stronger bed-nets made up of CYC cloth or blankets will be suitable for the forest workers.

“The bed-nets they gave were not suitable to use in the forest. It will tear if it hooks with branches of tree because it was made up of cotton. So it is only suitable for using at home but not for forest. I think that it would be better to use in the forest if it is made up of CYC cloth”.

(A 47-year-old male, Twin-nge Village)

Comment [G1]: To be delet

My husband never took bed-net to the forest. In my opinion, if a blanket is impregnated, it will be convenient for forest workers.”
(A 32- year-old female, Si-kar Village)

Most of the participants in Twin-nge Village reported that they were using ITNs with thanks for delivering free-of-charge without complaints. However, most of female participants from Si-kar Village discussed about the unfavorable characteristics of ITNs as reasons for their intermittent use or non-compliance. Some reported problems of ITNs such as weakness in sewing stitching lines, weak cloth, small size, pale color and unsuitable for using in the forest as reasons for their non-compliance.

Socio-cultural barriers affecting the utilization of ITNs

No serious socio-cultural barrier was reported by participants. However, some participants reported that it was not appropriate for older male and female children to sleep under the same bed-net.

“Sleeping together with parents and breast feeding child is not a problem. However, older male and female children should sleep separately with different bed-nets.”

(A 40-year-old male, Twin-nge Village)

DISCUSSION

This study investigated the community perception, acceptance and utilization of ITN in the selected rural communities. In this study, the main reason for ITN acceptability was effectiveness in killing mosquito and other insects. Moreover, free availability, mosquito nuisance, and popularity were also associated with bed-net use. A study done in Southern Ethiopia reported that the fact that ITN can prevent from mosquito bite was associated with utilization of ITNs [6]. In a north-east India study, usage of ITN was associated with protective effect not only against the mosquito bites but also against other household insects [7]. In line with previous research, ITN use is motivated by the co-

existence of a variety of reasons such as free distribution, mosquito density, nuisance and malaria burden [4,8]. This study also revealed that forest related works and evening social activities impact on the use of ITN. Similar finding was also reported in a study done in Solomon Islands. It revealed that climate, work and evening activities have an impact on the use of bed-nets [9].

In our study, general problems reported with the use of ITNs were weak in strength of cloth, easy to tear, small in size, pale in color, inadequate number of bed-nets required for age and sex composition of the family, not appropriate for forest related workers, and side effects such as dizziness, itchiness and burning sensation on face. A study done in Central Kenya reported that color, shape and affordability were some of the key potential factors which determined choice, use and acceptance of bed-nets [5]. In Nagaland and Mizoram, about 15-20 per cent ITN users reported to have suffered from skin irritation/ burning sensation of short durations which prompted them to stop their children, especially infants and toddlers, from sleeping under ITNs [7].

Participants reported that minimal information was provided at the point of distribution of ITNs. There was still considerable confusion on the protective effect of ITNs, maintenance of ITNs, and side effects. Therefore, free distribution of adequate and better quality ITNs based on sex and age composition of household members and provision of information about ITNs will be important for improving compliance. Moreover, research on the effect of impregnating blankets should be carried out for forest workers.

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Antioxidant potential of *Curcuma zedoaria* Rosc. (နန္ဒင်းဖြူ)

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This study was conducted to investigate the antioxidant activity of ethanolic extract of *Curcuma zedoaria* Rosc. (နန္ဒင်းဖြူ). DPPH (2, 2-diphenyl, 1-picryl hydrazyl) radical scavenging activity and total phenolic content of the extract were quantified. The extract exhibited free radical scavenging potential with IC₅₀ value of 24.50 µg/ml for DPPH, comparable with that of standard butylated hydroxytoluene (IC₅₀=24.48 µg/ml). The total phenolic content was found to be 27.29±2.90 mg/g extract. There was a significant positive correlation between the total phenolic content of the ethanolic extract and its DPPH scavenging activity with correlation coefficient of 0.934. The results obtained in the present study indicate that *Curcuma zedoaria* Rosc. is a potential source of natural antioxidants.

INTRODUCTION

The common free radicals are reactive oxygen species (ROS) namely superoxide radical, hydroxyl radical, and peroxy radical. These can be internally produced by cellular metabolism, inflammation by immune cells and externally by radiation and toxic chemicals. These free radicals can cause damage to parts of cells such as proteins, DNA, and cell membranes. Oxidation of polyunsaturated fatty acid in biological membranes by these ROS leads to the onset of many diseases such as coronary heart diseases, diabetes, Alzheimer's disease, cancer and cirrhosis [1].

Antioxidants are compounds that scavenge free radicals by preventing the onset and/or retarding the propagation of oxidizing chain reactions. There are two basic categories of antioxidants; synthetic and natural ones. The most commonly applied antioxidants are synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Restriction on use of synthetic antioxidants is being imposed because of their carcinogenicity [2-3]. Therefore, attention is now focused on the natural antioxidants. Antioxidants are polyphenol compounds [4], which are found in

all plants and in all parts of the plants (roots, tree bark, stalks, leaves, fruits, and seeds) [5]. *Curcuma zedoaria* Rosc. (နန္ဒင်းဖြူ) belongs to the family Zingiberaceae. Zingiberaceae plants have high medicinal value and have been used in traditional medicine for centuries. *Curcuma zedoaria* has been extensively cultivated as a vegetable, spice and perfume in South and Southeast Asian countries. The rhizomes of *Curcuma zedoaria* are widely used in the treatment of ulcers, wounds, tumours, atherosclerosis and inflammation and it is prescribed in various traditional preparations used for the treatment of Ohyl syndrome, which is caused by blood stagnation. In China, it is used clinically for treatment of several types of tumours [6].

The aim of this study was to assess the antioxidant activity of the ethanolic extract of *Curcuma zedoaria* Rosc. regarding to the free radical DPPH (2,2-diphenyl, 1-picrylhydrazyl) as well as its total phenolic content.

MATERIALS AND METHODS

Chemicals and reagents

Ascorbic acid, BHT, Folin-Ciocalteu reagent, gallic acid (GA), and DPPH were

purchased from Sigma Chemical Company (St. Louis MO, USA). Analytical grade ethanol, methanol, chloroform and petroleum ether were obtained from Merck (Darmstadt, Germany).

Preparation of the extract

Rhizomes of *Curcuma zedoaria* Rosc. were washed thoroughly with tap water, cut cross-sectionally into approximately 1 mm thickness, and air-dried. They were then finely powdered by using an analytical mill and weighed by using an analytical balance. They were then dissolved in 95% ethanol for two weeks at room temperature with intermediate shaking. The extract was filtered through the filter paper into evaporating basin, made evaporation, and weighed again to calculate the yield. Aqueous, petroleum ether, and chloroform extracts were also prepared for qualitative phytochemical screening.

Phytochemical screening

The aqueous, ethanolic, petroleum ether, and chloroform extracts of *Curcuma zedoaria* Rosc. were screened for the following constituents: proteins, cyanogenic glycosides, reducing sugars, non-reducing sugars, saponins, starch, carbohydrate, tannins, alkaloids, resins, α -amino acids, glycosides, phenolic compounds, flavonoids, terpenoids, steroids, and resinified volatile oil.

Free radical scavenging activity

Free radical scavenging activity of the ethanolic extract of *Curcuma zedoaria* Rosc. was measured by DPPH using the method of Molyneux [7]. Briefly, DPPH solution (60 μ M) was prepared in 95% ethanol and 2 ml of this solution was mixed with 2 ml of the various concentrations of samples of extracts (0.625 μ g/ml, 1.25 μ g/ml, 2.5 μ g/ml, 5 μ g/ml, 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml, 50 μ g/ml, 60 μ g/ml, 70 μ g/ml, 80 μ g/ml, 90 μ g/ml and 100 μ g/ml). After incubation at room temperature in the dark for 30 minutes, absorbance of the mixture was measured at

518 nm against 95% ethanol as blank using UV-visible spectrophotometer (UV-1601, Shimadzu). BHT and ascorbic acids were used as positive controls. The activities of the samples were evaluated by comparison with a control (containing 2 ml of DPPH solution and 2 ml of 95% ethanol). Each sample was measured in triplicate and averaged. The activity was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_c - A_s) / A_c] \times 100$$

where A_c is absorbance of the control and A_s is absorbance of the test solution.

Then, % inhibitions were plotted against log concentration of each of the test sample. Then, IC₅₀ (inhibitory concentration 50%) was calculated from the graph.

Total phenolic content

Total phenolic content in ethanolic extract was determined by using Folin-Ciocalteu reagent and external calibration with GA [8]. Briefly, 1 ml of the extract solution was added to 1ml of Folin-Ciocalteu reagent in a test tube and the contents were mixed thoroughly. After 5 minutes, 2 ml of 20% sodium carbonate were added to stand for 1 hour at room temperature in the dark. Absorbance was measured at 750 nm by using UV-visible spectrophotometer (UV-1601, Shimadzu). Total phenolic content was calculated by using GA calibration curve. Each sample was measured in triplicate and averaged. The results were expressed as mg GA/g dry material.

Statistical analysis

Results were expressed as mean $\pm$ standard deviation of three replicates. Experimental analyses were performed by Statistical Package for Social Sciences (SPSS) software version 11.5 (SPSS Inc. Chicago, IL). Statistical analysis was done with Student's t-test. Regression analyses for curve fits were also done. Pearson's correlation coefficient was also calculated for bivariate analysis. p value <0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

A qualitative phytochemical screening of aqueous, ethanolic, petroleum ether, and chloroform extracts of *Curcuma zedoaria* was performed. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if a definite heavy precipitate or flocculation was produced (Table 1).

Table 1. Phytochemical constituents of *Curcuma zedoaria* Rosc. extracts

Phytochemical constituents	<i>Curcuma zedoaria</i> Rosc. extracts	
α - amino acid	+	(Ethanolic extract)
Alkaloid	+++	(Aqueous extract)
Carbohydrate	++	(Aqueous extract)
Cyanogenic glycosides	-	(Aqueous extract)
Flavonoids	-	(Ethanolic extract)
Glycosides	+	(Ethanolic extract)
Non-reducing sugar	+++	(Aqueous extract)
Phenols	++	(Ethanolic extract)
Proteins	-	(Aqueous extract)
Reducing sugar	++	(Aqueous extract)
Resins	+	(Ethanolic extract)
Resinified volatile oil	+	(Petroleum ether extract)
Saponin	-	(Aqueous extract)
Starch	-	(Aqueous extract)
Steroids	+	(Chloroform extract)
	+	(Petroleum ether extract)
Tannin	-	(Aqueous extract)
Terpenoid	+	(Chloroform extract)

+ = Present, - = Absent

Free radical scavenging activity

Scavenging effect of *Curcuma zedoaria* ethanolic extract on DPPH was observed. DPPH is characterized as a stable free radical by virtue of delocalization of the spare electron over the molecule as a whole. The delocalization gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 518 nm. When a solution of DPPH was mixed with that of a substance that can donate a hydrogen atom, DPPH became reduced form with the loss of this violet color. The absorbance decreased when

DPPH was scavenged by an antioxidant. IC₅₀ value denotes the concentration of the sample required to scavenge 50% of the DPPH free radicals.

The scavenging effects of *Curcuma zedoaria* extract, BHT, and ascorbic acid are shown in Fig. 1.

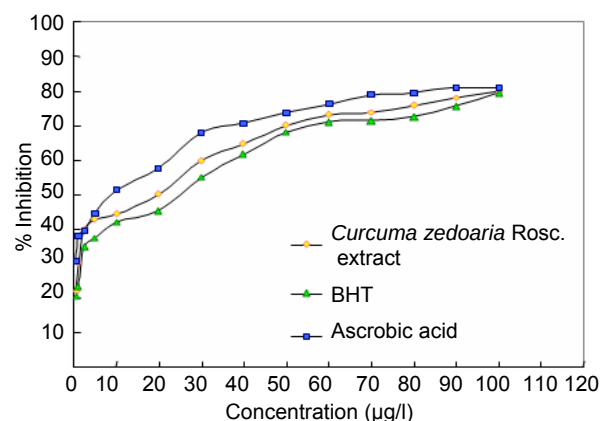


Fig. 1. Scavenging effects of *Curcuma zedoaria* extract, BHT, and ascorbic acid on DPPH radical

The ethanolic extract of *Curcuma zedoaria* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition at a concentration of 24.50 µg/ml. This value was comparable with that of BHT (IC₅₀=24.48 µg/ml), a potent synthetic antioxidant. IC₅₀ of ascorbic acid was found to be 7.68 µg/ml. IC₅₀ values were with statistical significance $p < 0.05$ and had high regression coefficients of $R^2 = 0.960$, $R^2 = 0.950$, and $R^2 = 0.972$ for *Curcuma zedoaria* Rosc., BHT, and ascorbic acid, respectively. Compared to the standards, the extract exhibited a similar curve of antioxidant activity. This result demonstrated that *Curcuma zedoaria* Rosc. extract has inhibitory activity against the DPPH radical.

Total phenolic content

Total phenolic content of *Curcuma zedoaria* was found to be 27.29 ± 2.9 mg/g extract.

Correlation between total phenolic content and antioxidant activity

Fig. 2 shows the correlation between the total phenolic content of *Curcuma zedoaria*

and its antioxidant activity. There is a significant positive relationship between total phenolic content and antioxidant activity ($r=+0.934$, $p<0.01$). Phenolic compounds are effective hydrogen donors, making the extract a very good antioxidant.

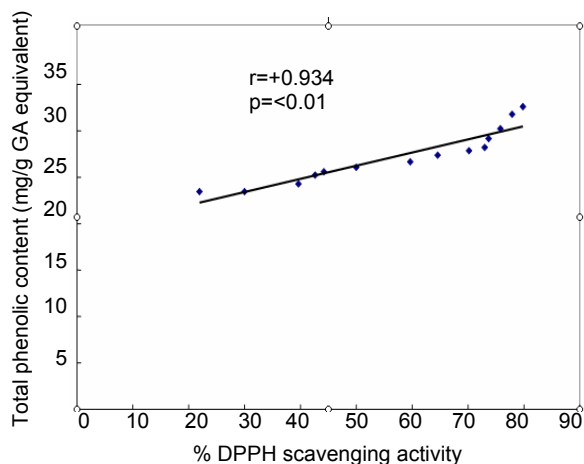


Fig. 2. Correlation between the total phenolic content and antioxidant activity of *Curcuma zedoaria* Rosc. extract

Conclusion

This study suggested that ethanolic extract of *Curcuma zedoaria* Rosc. possesses antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases.

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Glucose-6-phosphate dehydrogenase enzyme deficiency in Kayah and Rakhine states

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The aim of the study was to detect glucose-6-phosphate dehydrogenase (G-6-PD) enzyme deficiency in healthy male and female subjects in Kayah and Rakhine states. In male subjects, G-6-PD enzyme deficiency was detected by a qualitative test i.e., methaemoglobin reduction test (Brewer's test), followed by a semiquantitative test of agarose gel electrophoresis. For detection of enzyme deficiency in female subjects, the cytochemical staining method of Cornelis, Van-Nooden and Vogels was used. It is a method for staining G-6-PD activity in individual erythrocyte. The study was done in subjects who lived in Demowsoe of Kayah State and Sittway of Rakhine State during the period of April and May 2010. In Kayah State, out of 152 male subjects, 1.3% (n=2) were mild deficient and no severely deficient person was detected. In 252 female subjects, 0.41% (n=1) was mild deficient and 1.98% (n=5) were severely deficient. In Rakhine State, 151 male subjects were recruited and out of these patients 3.97% (n=6) were mild deficient and in 250 female subjects, 0.8% (n=2) were mild deficient. The subject with severe enzyme deficiency was not found. In treatment of malaria infection, the antimalarial drug primaquine can cause haemolysis in G-6-PD enzyme deficient patients. The finding of this study may be useful for primaquine treatment of hypnozoite stage in *Plasmodium vivax* and gametocyte stage in *Plasmodium falciparum* malaria infections.

INTRODUCTION

Genetic deficiency of glucose-6-phosphate dehydrogenase enzyme (G-6-PD), with consequent impairment of the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) is common in populations of Mediterranean and Afro-Caribbean origin. The defect is manifested as red cell haemolysis when susceptible individuals are subjected to oxidants, such as the anti-malarial primaquine, aspirin, or sulfonamides or when they have eaten fava beans [1]. G-6-PD catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate concurrent with reduction of NADP to NADPH, which in turn acts through glutathione and catalase pathways to detoxify hydrogen peroxide, thus counteracting oxidative stress to the cell. In the body, red cells are susceptible to oxidative damage because oxygen radicals

are generated continuously as haemoglobin cycles from deoxygenated to oxygenated form, as well as being readily exposed to exogenous oxidizing agents present in the blood. Hence, G-6-PD deficiency is the prototype cause of haemolytic anaemia due to intrinsic red cell enzyme abnormality [2].

G-6-PD enzyme activity is classified according to WHO classification [3]. Class I includes severely deficient variants that are associated with chronic non-spherocytic haemolytic anaemia. Class II variant has less than 10% of residual enzyme activity, but not are associated with chronic spherocytic haemolytic anaemia. Class III variants are moderately deficient (10-60% of enzyme activity), Class IV has normal enzyme activity and in Class V the enzyme activity is increased. In Myanmar, there are 3 types of G-6-PD variants: Gd^{B+} has normal enzyme activity, Gd^{B-} has 10-60% enzyme activity and Gd^{Myanmar} has less than 5% of

enzyme activity [4]. In the treatment of malaria infection today, primaquine is the most effective 8-amino-quinoline drug against *Plasmodium vivax* and *Plasmodium ovale* by destroying their exoerythrocytic forms, thus preventing relapse, and against *Plasmodium falciparum* by destroying the gametocytes and thereby inhibiting sporogony [5].

Primaquine is dangerous with G-6-PD deficiency and should not be used without knowledge of G-6-PD status or during pregnancy [6]. In Southeast Asia, the prevalence of G-6-PD deficiency differs greatly by region and ethnic group. In Myanmar, out of main 8 races, data of G-6-PD enzyme deficiency of 6 national races were already obtained. Due to importance of G-6-PD status in malaria treatment, the G-6-PD enzyme activity of remaining two national races is needed to be investigated.

Objectives

- To find out the G-6-PD enzyme deficiency in healthy male and female subjects of Kayah and Rakhine races
- To classify the G-6-PD enzyme activity in G-6-PD enzyme deficient subjects according to WHO classification.

MATERIALS AND METHODS

The cross-sectional descriptive study was done in Demowsoe Township of Kayah State and Sittway Township of Rakhine State from April to May 2010. All the subjects were unselected, unrelated, apparently healthy adult males and females more than 18 years of age. In Kayah State, 152 male subjects and 252 female subjects were recruited. In Rakhine State, 151 male subjects and 250 female subjects were recruited.

In male subjects, detection of G-6-PD enzyme deficiency was done by a qualitative test i.e., methaemoglobin reduction test [7] followed by a semiquantitative test i.e., agarose gel electrophoresis [4]. The agarose gel electrophoresis revealed three

genotypes in the subjects namely Gd^{B+} , Gd^{B-} and $Gd^{Myanmar}$. After staining with enzyme substrate solution, Gd^{B+} showed a deep purplish blue colour (normal enzyme activity, Class IV of WHO classification) and Gd^{B-} type showed a faint colouration (mild deficient type, 10-60% enzyme activity, Class III of WHO classification) while $Gd^{Myanmar}$ showed no band (severely deficient type, less than 5% of enzyme activity, Class II of WHO classification) [4].

Female subjects were differentiated to homozygote and heterozygote by using the sensitive cytochemical staining method for G-6-PD activity in individual erythrocytes [8]. In that method, considerable formazan production occurs in normal erythrocytes. In a healthy person, considerable formazan production occurs in most of the erythrocytes and only a small percentage of the cells appeared to be negative. Two cells populations of one completely negative and other with a variable amount of formazan per cell in almost equal numbers would be discerned in heterozygote for G-6-PD deficiency. Homozygous deficiency leads to a population of negative cells with a few positive ones after staining.

Diagnosis of G-6-PD enzyme deficiency

(for male subject)

Methaemoglobin reduction test (Brewer's test)

Normal

Deficient

Agarose gel electrophoresis

Gd^{B+}

Gd^{B-}

$Gd^{Myanmar}$

Deep purplish
blue colour
(Normal)

Faint
blue colour
(Mild deficient)

No band
(Severely
deficient)

(for female subject)

Cytochemical staining method

Most of the
erythrocytes
contain
formazan
colour

(Normal)

In two cells popula-
tion, one completely
negative and other
with a variable
amount of formazan
per cell, almost
equal in number

(Heterozygous
deficiency)

A population
of negative
cells with a
few positive
ones.

(Homozygous
deficiency)

The ethical clearance was obtained from The Institutional Ethical Review Committee, Department of Medical Research (LM).

RESULTS

In Kayah State, total 404 subjects were recruited, 152 males and 252 females. In these patients, 0.13% (n=2) of male subjects were mild deficient and severely deficient subjects were not detected (Table 1).

Table 1. Results of methaemoglobin reduction test (Brewer's test) and 1.2% agarose gel electrophoresis in G-6-PD enzyme deficient male subjects of Kayah State

Code no.	Methaemoglobin reduction test	Agarose gel electrophoresis (Band density)	G-6-PD variants	WHO classification of G-6-PD enzyme activity
KM 85	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60%)
KM102	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60%)

Table 2. Results of G-6-PD enzyme deficient female subjects in Kayah State

Code no.	% of stained erythrocytes	Mild/ Severe	G-6-PD variants	WHO classification of G-6-PD enzyme activity
KF 66	8.90	Severe	Gd ^{B-} Myanmar	Class II (<10)
KF 73	9.20	Severe	Gd ^{B-} Myanmar	Class II (<10)
KF 155	9.80	Severe	Gd ^{B-} Myanmar	Class II (<10)
KF 219	8.40	Severe	Gd ^{B-} Myanmar	Class II (<10)
KF 223	9.50	Severe	Gd ^{B-} Myanmar	Class II (<10)
KF 225	58	Mild	Gd ^{B-}	Class III (10-60)

Table 3. Results of methaemoglobin reduction test (Brewer's test) and agarose gel electrophoresis in G-6-PD deficient male subjects of Rakhine State

Code no.	Methaemoglobin reduction test	Agarose gel electrophoresis (Band density)	G-6-PD variants	WHO classification of G-6-PD enzyme activity (%)
RM 1	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
RM 4	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
RM 9	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
KHAM 33	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
GHAM27	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
GHAM29	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)

Table 4. Results of cytochemical staining method in G-6-PD deficient female subjects in Rakhine State

Code no.	Percentage of stained erythrocytes	Mild/ Severe	G-6-PD variants	WHO classification of G-6-PD enzyme activity (%)
RF 68	58%	Mild	Gd ^{B-}	Class III (10-60)
RF 75	55%	Mild	Gd ^{B-}	Class III (10-60)

In 252 female subjects, 0.41% (n=1) was mild deficient and 1.98% (n=5) were severely deficient (Table 2).

In Rakhine State, 401 subjects were recruited, 151 were males and 250 were females. In these patients, 3.97% (n=6) of male subjects were mild deficient and severely deficient ones were not found (Table 3). In 250 female subjects, 0.8% (n=2) were mild deficient and severely deficient ones were not found in this study (Table 4).

DISCUSSION

Primaquine (8-aminoquinoline) is the only available drug for preventing relapse of malaria. The risk of relapse of *Plasmodium vivax* malaria without primaquine therapy ranged from 5% to 80% or more depending largely upon geographic location [8]. Primaquine is also used as a very potent gametocytocidal drug in *Plasmodium falciparum* infection. Because of the unpleasant haemolysis side effect of primaquine, many medical practitioners are reluctant to use the primaquine in patients of unknown G-6-PD enzyme activity.

In Myanmar, many researchers and scientists carried out the research on the prevalence of G-6-PD enzyme deficiency. Its prevalence in male was 9% in Bamar, 4% in Mon, 14% in Kayin, 8% in Kachin and overall incidence was 9% [9]. In another study, its prevalence rate was 5.3% in Bamar, 3.3% in Mon, 3.3% in Shan, 6.7% in Kayin, 3.2% in Indian and 3.2% in Chinese [10]. In 2009, our group detected the G-6-PD enzyme deficiency in Chin race. In Chin race 4.76% of mild deficiency and 0.68% of severe deficiency were detected in

male subjects and in female subjects, mild deficiency was 2.68% and severe deficiency was 0.38% [11].

In this study, total 404 subjects of Kayah race were recruited, 152 males and 252 females. Some 0.13% (n=2) of male subjects were mild deficient (Class III in WHO classification) and severely deficient subjects were not detected. In 252 female subjects 0.41% (n=1) was mild deficient (WHO Class III) and 1.98% (n=5) were severely deficient (WHO class II). In Rakhine State, 401 subjects were recruited, 151 were males and 250 were females. In these patients, 3.97% (n=6) of male subjects were mild deficient (Class III of WHO classification) and severely deficient were not found. In 250 female subjects, 0.8% (n=2) were mild deficient (WHO Class III) and no cases of severely deficient was found.

In our country, there are eight main national races and G-6-PD enzyme deficiency data of each race had been obtained. In Kayah State, five out of six female subjects had severe deficiency state (<10% of stained erythrocytes) and G-6-PD deficient female subjects had mild state (10-60% of stained erythrocytes) in Rakhine State. Because of the limited laboratory facilities and endemic malaria infection in remote areas, the above findings will be useful for the officials of vector borne diseases control in radical treatment of *Plasmodium vivax* infection and blockage of transmission in *Plasmodium falciparum* infection.

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Kayah and Rakhine states and last, not the least, is our subjects for their kind and helpful co-operation.

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