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## Bacterial isolates from non-processed fruit juices of Yangon

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A total of 70 samples of 7 different types of non-processed fruit juices (tamarind, water melon, sugar cane, pine apple, papaya, lime and black agar) and 15 different processed samples were collected randomly from six townships of Yangon from October 2002 to March 2004. All the samples of pine apple, water melon, papaya, sugar cane, and black agar drinks examined in the current study were found to be contaminated with coliforms (100%). In contrast, 40% and 10% of tamarind juice samples were contaminated with coliforms and faecal coliforms respectively. All samples of papaya, sugar cane and black agar juices were contaminated (100%) with faecal coliforms, while pine apple juice showed only 70% contamination. *Escherichia coli*, *Klebsiella aerogenes*, *Salmonella spp.*, *Shigella boydii*, *Proteus mirabilis*, *Vibrio cholerae*, *Staphylococcus epidermidis* and *Staphylococcus aureus* were isolated and identified from the non-processed soft drinks. However, no bacteria was isolated from lime juice samples. The water that was used for preparation of the drinks was found to be contaminated with bacterial pathogens. The antibiotic susceptibility patterns of the isolated enteropathogenic *E. coli* (EPEC) showed that they were resistant to ampicillin and carbenicillin (63.64% each), and *Vibrio* species to ampicillin (80%). Plasmids were isolated from 16 out of 32 *E. coli* and 14 out of 15 *Vibrio* species. The size of plasmids was estimated ranging from 20 to 23.13 kilo base pairs.

## INTRODUCTION

Ingestion of contaminated food or water is considered to be the principle mode of transmission of enteric pathogens [1]. Some bacterial species are always present and may constitute the normal flora of natural water. Opportunistic bacterial pathogens, naturally present in the environment are not usually regarded as pathogens but are able to cause diseases in people with impaired local or general immune mechanism. Safe water must be free from microorganisms and chemical substances. Fruits and vegetables can also be contaminated in areas where the lands are fertilized with human faeces [2]. Much attention had been paid to the quality, contamination and spoilage of soft drinks [3]. Contamination may occur through various sources from vending machines until they are

sold to consumer. Bacteria can survive in ice of popular drinks [4]. Standard quality control protocols should be implemented for drinking water distribution networks [5] and relationship of drinking water quality and infectious diarrhoeal diseases was also reported [6,7]. The present study was carried out to demonstrate the occurrence of bacterial contamination of locally made, non-processed soft drinks in Yangon area, Myanmar. The current research was undertaken to isolate and identify bacteria from non-processed and processed local soft drinks of Yangon, Myanmar to help in food quality control and safety.

The specific objectives were:

- to detect the presence of coliforms and faecal coliforms in the processed and non-processed local soft drinks.

- to determine the distribution pattern of bacteria in the soft drinks tested township-wise. and
- to identify the serotypes and the antibiotic susceptibility of the isolated bacterial strains and to do the plasmid analysis of the bacterial isolates.

## MATERIALS AND METHODS

Period of study was from October 2002 to March 2005.

### *Collection of samples*

Seventy different non-processed drink samples (tamarind, water melon, sugar cane, pine apple, papaya, lime and black agar) and 15 different processed soft drinks (tamarind juices, lychee, milk juices, and soybean milk) were collected without ice from shops and vendors located at various townships (Hlaing, Insein, Kamayut, Lanmadaw, Tamwe and Sanchaung) of Yangon area.

### *Determination of coliforms and faecal coliforms in the soft drinks*

The presence of coliforms and faecal coliforms was determined by multiple tube method and by membrane filtration technique as described in WHO, 1997 [2].

### *Isolation, identification and characterization of bacteria from the soft drinks*

Based on the methods described in WHO [8], total bacterial counts and specific bacterial counts were determined. The bacterial isolates were identified based on the biochemical and serological tests [9].

### *Serological characterization of bacterial isolates*

Slide agglutination tests were carried out using respective antisera of *E. coli*, *Salmonella*, *Shigella* and *Vibrio* spp., provided by Denka-Seika Company Limited, Japan, Difco, USA or DMR-001.

### *Antibiotic susceptibility testing*

All EPEC isolates and *Vibrio* isolates obtained were subjected to antibiotic susceptibility testing by the method of Kirby-Bauer [10].

### *Plasmid DNA analysis of bacterial isolates*

Rapid isolation of plasmid DNA was carried out by a modified Birnboim procedure [11] as described by Schleif and Wensink [12].

## RESULTS

### *Processed soft drinks*

Pathogenic bacteria was not isolated from all test samples.

### *Presumptive total coliform counts from the non-processed soft drinks samples*

All the samples of water melon, papaya, black agar and pine apple juices were contaminated with coliforms. The coliform bacteria were absent only in lime juices. In contrast, they were all very high in papaya, water lemon, black agar and sugar cane juices (Table 1). The coliform counts in water melon, papaya, black agar, pine apple and sugar cane juices did not show any significant differences, but the counts in tamarind and lime juices when compared with previous samples showed significant differences ( $p < 0.04$ ).

### *Presumptive faecal coliform (FC) count from the non-processed soft drinks samples*

All water melon, papaya, black agar and sugar cane samples (100 percent) were contaminated with faecal coliforms. Faecal coliforms were identified from 3 samples of pine apple. In contrast, only one sample of tamarind juice was contaminated with faecal coliforms. Faecal coliforms were absent in all the 10 samples of lime juice. The faecal coliforms counts from all different samples of juices ranged from 6 to >1800 MPN/100ml (Table1). Similar findings were obtained as in coliform count and the counts in pine apple, tamarind and lime were

found to be significantly different ( $p < 0.04$ ) when compared to the counts from water melon. The water samples used for the preparation of the juices were found to be contaminated with coliforms (85.7%) and also with faecal coliforms (33.3%).

#### *Distribution of bacterial isolates in the non-processed soft drinks*

Presence of *E. coli* was detected in 71.4% of non-processed soft drink samples and 85.7% of water samples used for preparation of the juices from various shops. *Klebsiella aerogenes* was found in 12 samples (17.1%), *Staphylococcus aureus* in 8 samples (11.4%), *Staphylococcus epidermidis* in 6 samples (8.57%). *Salmonella* species in one sample (1.4%), and *Vibrio cholerae* in 5 samples (7.1%) of the non-processed drinks. *Shigella boydii* was isolated from 3 samples (4.3%) of juices and only one sample (4.8%) of water which was used to make the soft drinks was contaminated with *Shigella* species (Table 2).

Table 1. Presumptive total coliform and faecal coliform counts in non-processed soft drinks samples

| Sr. No. | Fruit juices soft drinks | Number of samples contaminated with coliforms and faecal coliforms |                  | Range of coliform and faecal coliform counts (MPN/100ml) |                  |
|---------|--------------------------|--------------------------------------------------------------------|------------------|----------------------------------------------------------|------------------|
|         |                          | Coliforms                                                          | Faecal coliforms | Coliforms                                                | Faecal coliforms |
| 1       | Tamarind (n=10)          | 4                                                                  | 1                | 6-84                                                     | 6-9              |
| 2       | Water melon (n=10)       | 10                                                                 | 10               | >1800                                                    | >1800            |
| 3       | Papaya (n=10)            | 10                                                                 | 10               | >1800                                                    | 6- >1800         |
| 4       | Black agar (n=10)        | 10                                                                 | 10               | >1800                                                    | 12- >1800        |
| 5       | Pine apple (n=10)        | 4                                                                  | 3                | 12- >1800                                                | 6- >1800         |
| 6       | Sugarcane (n=10)         | 10                                                                 | 10               | >1800                                                    | 6- >1800         |
| 7       | Lime (n=10)              | 0                                                                  | 0                | <2                                                       | <2               |
| 8       | Total (n=70)             | 48<br>68.56 %                                                      | 44<br>8.56 %     | <2- >1800                                                | <2- >1800        |
|         | Water (n=21)             | 18<br>(85.71%)                                                     | 7<br>(33.33%)    | 12- >1800                                                | 6- >1800         |

Table 2. Bacteria isolates from various kinds of non-processed soft drinks

| Name of non-processed soft drinks | <i>Escherichia coli</i> | <i>Klebsiella aerogenes</i> | <i>Salmonella</i> a species | <i>Shigella boydii</i> | <i>Proteus mirabilis</i> | <i>Vibrio cholerae</i> | <i>Staphylococcus epidermidis</i> | <i>Staphylococcus aureus</i> | Total bacterial isolates |
|-----------------------------------|-------------------------|-----------------------------|-----------------------------|------------------------|--------------------------|------------------------|-----------------------------------|------------------------------|--------------------------|
| Tamarind (n=10)                   | 4                       | 2                           | 0                           | 1                      | 0                        | 2                      | 1                                 | 0                            | 8                        |
| Water melon (n=10)                | 9                       | 0                           | 0                           | 0                      | 2                        | 2                      | 2                                 | 2                            | 17                       |
| Papaya (n=10)                     | 10                      | 1                           | 0                           | 1                      | 1                        | 0                      | 2                                 | 2                            | 17                       |
| Black agar (n=10)                 | 10                      | 2                           | 1                           | 0                      | 0                        | 1                      | 0                                 | 1                            | 15                       |
| Sugar cane (n=10)                 | 9                       | 5                           | 0                           | 1                      | 1                        | 0                      | 1                                 | 3                            | 20                       |
| Pine apple (n=10)                 | 8                       | 2                           | 0                           | 0                      | 1                        | 0                      | 0                                 | 0                            | 11                       |
| Lime (n=10)                       | 0                       | 0                           | 0                           | 0                      | 0                        | 0                      | 0                                 | 0                            | 0                        |
| Total (n=70)                      | 50<br>(71.43)           | 12<br>(17.14)               | 1<br>(1.43)                 | 3<br>(4.28)            | 5<br>(7.14)              | 5<br>(7.14)            | 6<br>(8.57)                       | 8<br>(11.43)                 | 88                       |
| Water (n=21)                      | 18<br>(85.71)           | 1<br>(4.76)                 | 0                           | 1<br>(4.76)            | 2<br>(2.86)              | 0                      | 0                                 | 0                            | 22                       |

Figures in parenthesis denote percentages of the bacterial isolates in non-processed soft drinks

The bacterial isolates that were identified from the non-processed soft drinks obtained from different localities of the Yangon city are detailed in Table 3. Serology showed that of 125 isolates of *E. coli*, 12 different types of *E. coli* were serogrouped; out of

these, 7 isolates were O55K59, 5 isolates were O128K67, and 3 isolates were O26K60. The other serogroups were: O6K15, O28K73, O78K80, O114K90, O126K71, O127K63, and O144Kx2 (Table 3).

Table 3. Places of collection and species of bacterial detected in non- processed fruit juices and water used for preparation

| Place          | Tamarind<br>n=10                                                                      | Water melon<br>n=10                                                                                  | Sugarcane<br>n=10                                                                                | Pineapple<br>n=10                           | Papaya<br>n=10                                                                       | Black agar<br>n=10                                                                                                           | Water<br>n=21                                                           |
|----------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Hledan         | <i>E. coli</i> (2)<br>O6K15, O28K73<br><i>Klebsiella</i> spp.<br><i>S.epidermidis</i> | <i>E. coli</i> (2)                                                                                   | <i>E. coli</i> (4)<br>O26K60,<br>O55K59<br>O128K67,<br><i>Klebsiella</i> (3)<br><i>S. aureus</i> | <i>E. coli</i> (3)<br><i>Klebsiella</i> (2) | <i>E. coli</i>                                                                       | <i>E. coli</i> (2)<br><i>Klebsiella</i> spp.                                                                                 | <i>E. coli</i> (5)<br>O26K60                                            |
| Insein         | <i>E. coli</i>                                                                        | <i>E. coli</i><br><i>Proteus</i> spp.                                                                | <i>E. coli</i> (1),<br>O55K59<br>O128K67,<br><i>Proteus</i> spp.<br><i>Vibrio</i> spp.           | <i>E. coli</i> (2)                          | <i>E. coli</i><br><i>Klebsiella</i> spp.<br><i>S. aureus</i><br><i>S.epidermidis</i> | <i>E. coli</i> (2)<br>055K59,<br>0114K90<br>0144Kx2<br><i>Klebsiella</i> spp.<br><i>Salmonells</i> spp<br><i>Vibrio</i> spp. | <i>E. coli</i>                                                          |
| Tamway         | <i>Shigella</i> spp.<br><i>Vibrio</i> spp.                                            | NT                                                                                                   | NT                                                                                               | <i>E. coli</i>                              | <i>E. coli</i><br><i>Shigella</i> spp.<br><i>S.epidermidis</i>                       | <i>E. coli</i><br><i>S. aureus</i>                                                                                           | <i>E. coli</i> (2)<br><i>Shigella</i> spp.                              |
| Sinmaleik      | <i>Klebsiella</i> spp                                                                 | NT                                                                                                   | NT                                                                                               | <i>E. coli</i><br><i>Proteus</i> spp.       | NT                                                                                   | NT                                                                                                                           | <i>E. coli</i> (2)<br><i>Proteus</i> spp.                               |
| Hlaing-yadanar | NT                                                                                    | <i>E. coli</i> (4),<br>O55K59<br>O126K71,<br>O128K67<br><i>S.epidermidis</i> ,<br><i>Vibrio</i> spp. | NT                                                                                               | NT                                          | <i>E. coli</i> (2)<br>055K59(2)<br><i>Vibrio</i> (1)                                 | NT                                                                                                                           | <i>E. coli</i>                                                          |
| Kyundaw        | <i>Vibrio</i> spp.                                                                    | NT                                                                                                   | <i>E. coli</i> ,<br><i>Vibrio</i> spp.<br><i>S.epidermidis</i>                                   | NT                                          | not isolated                                                                         | <i>E. coli</i> (2)<br>026K60<br>055K59<br><i>Vibrio</i> spp.                                                                 | <i>E. coli</i> (2)                                                      |
| 10th mile      | <i>E. coli</i>                                                                        | <i>E. coli</i> ,<br>O128K67<br><i>S. aureus</i> ,<br><i>S.epidermidis</i><br><i>Vibrio</i> spp.      | <i>E. coli</i> ,<br><i>Vibrio</i> spp.<br><i>Klebsiella</i> (1),<br><i>Shigella</i> spp.         | not isolated                                | <i>E. coli</i><br><i>Vibrio</i> spp.(2)                                              | <i>E. coli</i>                                                                                                               | <i>E. coli</i> (2)<br>O78K80                                            |
| Myenigone      | NT                                                                                    | <i>Proteus</i> spp.<br><i>S. aureus</i> ,<br><i>Vibrio</i> spp.                                      | <i>E. coli</i> (2)<br><i>Klebsiella</i><br><i>S. aureus</i> (2)                                  | not isolated                                | <i>E. coli</i> (3).<br><i>Proteus</i>                                                | <i>E. coli</i><br>0128K67                                                                                                    | <i>E. coli</i> (2).,<br><i>Klebsiella</i><br>spp.<br><i>Proteus</i> spp |
| Lanmadaw       | NT                                                                                    | <i>E. coli</i><br>O127K63                                                                            | NT                                                                                               | <i>E. coli</i>                              | <i>E. coli</i> ,<br><i>S. aureus</i>                                                 | <i>E. coli</i> (2)<br>0128K67<br><i>Vibrio</i> spp                                                                           | <i>E. coli</i>                                                          |
| Total          | 10                                                                                    | 18                                                                                                   | 23                                                                                               | 11                                          | 20                                                                                   | 17                                                                                                                           | 22                                                                      |

NT=Not tested

### Antibiotic sensitivity/resistance of the bacterial isolates

Data show that most of the isolates of *E. coli* were resistant to ampicillin, carbenicillin and amikacin while they were very sensitive to chloramphenicol, trimethoprim and tetracycline. Most of the isolates of *Vibrio* spp. exhibited resistance to ampicillin, tetracycline and carbenicillin while they showed sensitivity to chloramphenicol, carbenicillin, trimethoprim and amikacin (Table 4).

### Plasmid profile

Plasmid analysis of 32 isolates of *E. coli* was done and plasmids were detected in 16 of the isolates while absent in others. All the plasmids were large in size (20-23kbp). Different strains of *E. coli* exhibited different sized plasmids probably reflecting the differences in the strains and also in fruit sources. The *E. coli* serotypes seemed to possess the largest sized plasmids. Altogether 16 isolates of *Vibrio* species were screened for the presence of plasmids

and 14 isolates carried plasmids. All plasmids were estimated to be similar in size (equal to or greater than 23.13 kbp). Isolates 6 and 9 have been isolated from different fruits and localities and they exhibited the presence of large sized plasmids.

Table 4. Antibiotic susceptibility of *E.coli* (n=33) and *Vibrio* species (n=15) isolated from non-processed soft drinks samples

| Susceptibility to antibiotics | Antibiotics |          |                 |               |                               |              |
|-------------------------------|-------------|----------|-----------------|---------------|-------------------------------|--------------|
|                               | Ampicillin  | Amikacin | Chloramphenicol | Carbenicillin | Trimethoprim/sulfamethoxazole | Tetracycline |
| <i>E. coli</i>                | 21          | 14       | 4               | 21            | 8                             | 3            |
| Resistant                     | (63.64)     | (42.42)  |                 | (63.64)       | (24.24)                       |              |
| Intermediate resistant        | 2           | 16       | 0               | 0             | 0                             | 15           |
| Moderately susceptible        | 8           | 0        | 0               | 4             | 22                            | 0            |
| Susceptible                   | 2           | 3        | 29              | 8             | 14                            | 15           |
| Total <i>E. coli</i>          | 33          | 33       | 33              | 33            | 33                            | 33           |
| <i>Vibrio</i> species         | 12          | 2        | 2               | 3             | 3                             | 5            |
| Resistant                     | (89.00)     |          |                 | (20.00)       | (20.00)                       | (33.33)      |
| Intermediate resistant        | 0           | 8        | 0               | 5             | 0                             | 8            |
| Moderately susceptible        | 1           | 0        | 0               | 1             | 3                             | 0            |
| Susceptible                   | 2           | 5        | 13              | 6             | 9                             | 2            |
| Total <i>Vibrio</i> species   | 15          | 15       | 15              | 15            | 15                            | 15           |

Figures in parenthesis denote percentages

## DISCUSSION

The present study has shown that none of the processed soft drinks were contaminated with bacteria indicating that all the 15 different processed soft drinks were processed properly.

In contrast, most of the non-processed soft drink samples tested in the current study had shown that 50-100% were contaminated with coliforms and 10-100% with faecal coliforms. *Escherichia coli* was isolated from 40% of the samples of tamarind juices

and from 80-100% of pine apple, water melon, sugar cane and papaya juices. Moreover, *E. coli* was isolated from 86% of water which was used to prepare the soft drinks. The reasons for these might have been due to the ubiquitous presence of *E. coli* in the localities and the hands and intestine of man; for example, about 200 to 400 million *E. coli* are reported to be excreted per day per person [13].

Other sources of contamination could be the handlers who prepare the drinks. They might have contaminated the drinks with *E. coli* or other coliforms or infectious bacteria. These might be one of the reasons for the products to be contaminated with *E. coli*. Surprisingly, *E. coli* could not be isolated from the freshly prepared lime juices probably due to the constituents present were antibacterial. Another reason for the low isolation rate of *E. coli* from tamarind juices and lime juices might be due to the low acidity of these drinks. The pH of tamarind juices ranges from 4-5 whereas the lime juices have a pH of 2. In contrast, the pH of other juices and soft drinks were in the range of 6-8. Most bacteria cannot thrive at the low pH level as most bacteria prefer a slightly alkaline pH of between 7.2 and 7.6 for their survival and proliferation [1, 13].

Plasmid analysis provides a useful tool, primarily to classify organisms at the subspecies level for epidemiologic investigations [14]. Plasmids of *E. coli* and *Vibrio cholerae* from this study showed similarities with those of the strains isolated from clinical sources. Thus, the spread of infectious bacterial in these juices might be due to the improper sanitation in that environment.

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**Energy expenditure of Myanmar elderly people from home for the aged (Hninsigone), Yangon.**

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To find out resting energy expenditure (REE) and total energy expenditure (TEE), the study was conducted on 25 apparently healthy Myanmar elderly people (11 males and 14 females) from Hninsigone home for the aged, Yangon. Stratified random sampling was used in accordance with age and sex. Energy cost of each activity was measured by indirect calorimetry method and time usage for each activity was recorded by minute-to-minute registration. REE were found to be  $1022.79 \pm 314.52$  kcal/d in males and  $848.06 \pm 378.69$  kcal/d in females. Both males and females spent half of the day lying/sleeping and one third of the day sitting. TEE were  $1812.27 \pm 334.80$  kcal/d in males and  $1484.70 \pm 480.96$  kcal/d in females and energy expenditure for physical activity (EEPA) were 608.25 kcal/d in males and 488.17 kcal/d in females. TEE were found to be 1.77 times REE in males and 1.75 times REE in females. It was found that the elderly people from our study had lower REE and TEE when compared to Myanmar adults and Caucasian elderly people.

## INTRODUCTION

In developed as well as developing countries, many elderly people are now being maintained in the community. Thus, the provision of health care to the elderly people should be an important aim of the health service. Among the problems concerned with care of the elderly people, those at age over 75 are at greatest risk for malnutrition [1]. However, there has been a limited information concerning energy requirement of elderly compared with other groups. Assessment of total energy expenditure is necessary to define suitable level of energy intake and energy expenditure for prevention and treatment of malnutrition and obesity and to determine appropriate recommendation on dietary needs of different population groups. Energy requirement of human can be determined by measuring energy intake during a period of stable body weight and

body composition. Energy intake can be estimated from dietary intake records. However, the current recommendation on energy requirements, based upon the studies of weighed food was significantly underestimated [2]. WHO/FAO/UNU recommended that the determination of energy requirement should be based upon direct measurement of energy expenditure performed under free-living condition.

Therefore, the present study aimed to find out total energy expenditure of Myanmar elderly persons from home for the aged in order to define suitable level of energy intake.

## MATERIALS AND METHODS

### *Subjects*

The study was conducted on 25 apparently healthy Myanmar elderly people (11 males

and 14 females) from Hninsigone home for the aged, Yangon. Stratified random sampling was used in accordance with age and sex. None of the subjects suffered from acute illness, overt diseases or gave history of endocrinopathy and all were able to walk and dress unaided.

#### *Anthropometric and body composition assessment*

Body weight was measured to the nearest 0.1 kg with bathroom scale weighing machine, calibrated with standardized weights and the weight was recorded. Body height was measured to the nearest 0.1 cm using a stadiometer. Body Mass Index (BMI) was calculated as weight / height<sup>2</sup> (kg/m<sup>2</sup>). Skin-fold thickness at biceps, triceps, sub-scapular and suprailiac were measured on the left side of the body using a Harpenden caliper by a well-trained technician. Body fat percent was calculated by using the formula described by Durnin and Rahaman, 1967 [3].

#### *Measurement of energy expenditure*

##### *1. Resting Energy Expenditure (REE)*

It was measured four hours after breakfast in the morning by respiratory gas analysis and by using a facemask system for breath collection. Flow rate was measured by Portable Respiratory Air Monitor (Harvard) and oxygen content of expired air was analyzed by Oxygen analyzer (Miniox, USA). Energy expenditure was calculated using RQ as 8.2 [4].

##### *2. Total Energy Expenditure (TEE)*

Energy cost of each activity per minute was measured during each activity by indirect calorimetry method mentioned above. Time usage for each activity was recorded by minute - to- minute registration. Recording was performed by trained research assistants who stayed with the subjects for two consecutive days from 6 am to 6 pm. Observation was done without awareness of observed individual. The recall method was used to determine activities carried out

when the assistants were not present. TEE was then calculated.

#### *Statistical analysis*

Data were expressed as mean  $\pm$  SD. Comparison was made using Student 't' test for unpaired samples (two tailed). Differences were considered significant if  $p < 0.05$ .

## **RESULTS**

Table 1 shows general characteristics of the subjects.

Table 1. General characteristics of the subjects

|                        | Male              | Female            |
|------------------------|-------------------|-------------------|
| Age (yr)               | 81.18 $\pm$ 4.28  | 81.00 $\pm$ 5.95  |
| Height (cm)            | 155.47 $\pm$ 3.98 | 146.38 $\pm$ 5.10 |
| Weight (kg)            | 45.63 $\pm$ 6.13  | 40.46 $\pm$ 6.88  |
| BMI                    | 18.9 $\pm$ 2.3    | 18.6 $\pm$ 3.3    |
| Body Fat Percent (%)   | 18.72 $\pm$ 3.36  | 27.35 $\pm$ 4.40  |
| Fat Mass (FM)          | 8.70 $\pm$ 2.55   | 11.20 $\pm$ 3.90  |
| Fat Free Mass (FFM)    | 36.93 $\pm$ 3.97  | 28.63 $\pm$ 3.68  |
| Number of subjects (n) | 11                | 14                |

From the time usage pattern of the elderly Table 2, it was observed that half of the day was occupied with lying/sleeping activities and one third of the day with sitting activities (Table 2).

Table 2. Time usage pattern of the elderly from Hninsigone home for the aged

| Activity                   | Male                | Female              |
|----------------------------|---------------------|---------------------|
| Lying / Sleeping (minutes) | 639.64 $\pm$ 80.79  | 620.11 $\pm$ 205.43 |
| Sitting (minutes)          | 464.05 $\pm$ 101.12 | 501.96 $\pm$ 88.35  |
| Standing (minutes)         | 139.45 $\pm$ 59.19  | 106.11 $\pm$ 46.81  |
| Walking (minutes)          | 150.73 $\pm$ 56.41  | 129.32 $\pm$ 42.96  |
| Others (minutes)           | 46.09 $\pm$ 13.15   | 47.07 $\pm$ 13.35   |

When the body composition and energy expenditure of elderly males and females were compared with those of adult Myanmar (Bamar) males and females [5]

respectively (Table 3), elderly male and female were found to have lower body weight, lower fat free mass, lower REE and TEE.

Table 3. Comparison of body composition and energy expenditure of elderly males and females with those of adult Bamar males and females from South Dagon Township, Yangon.

|                  | Male                |                   |           | Female             |                   |           |
|------------------|---------------------|-------------------|-----------|--------------------|-------------------|-----------|
|                  | Elderly (n=11)      | Adult (n=22)      | 'p' value | Elderly (n=14)     | Adult (n=16)      | 'p' value |
| Age (yr)         | 81.18<br>± 4.28     | 19-60             |           | 81.00<br>± 5.95    | 19-43             |           |
| Weight (kg)      | 45.63<br>± 6.13     | 51.96<br>± 6.02   | 0.007     | 40.46<br>± 6.88    | 45.94<br>± 6.04   | 0.02      |
| Body fat %       | 18.72<br>± 3.36     | 10.52<br>± 2.86   | 0.000     | 27.35<br>± 4.4     | 25.76<br>± 6.54   | 0.45      |
| REE (Kcal / min) | 1022.79<br>± 314.52 | 1906.3<br>± 77.12 | 0.012     | 848.06<br>± 378.69 | 1215.1<br>± 37.31 | 0.08      |
| TEE (Kcal / min) | 1812.27<br>± 334.8  | 2818<br>± 114     | 0.001     | 1484.7<br>± 480.96 | 2475<br>± 76      | 0.01      |

Table 4. Comparison of body composition and energy expenditure of Myanmar elderly males and females with those of elderly males and females from the United Kingdom (UK)

|                 | Elderly male        |                     |           | Elderly female     |                    |           |
|-----------------|---------------------|---------------------|-----------|--------------------|--------------------|-----------|
|                 | Myanmar n = 11      | UK n = 23           | 'p' value | Myanmar n = 14     | UK n = 10          | 'p' value |
| Age (yr)        | 81.18<br>± 4.28     | 82.00<br>± 3.00     | 0.521     | 81<br>± 5.95       | 73<br>± 3          | 0.051     |
| Weight (kg)     | 45.63<br>± 6.13     | 72.4<br>± 10.5      | 0.0001    | 40.46<br>± 6.88    | 60.00<br>± 7.2     | 0.0000    |
| Body fat %      | 18.72<br>± 3.36     | 30.40<br>± 4.60     | 0.0000    | 27.35<br>± 4.40    | 34.23<br>± 2.30    | 0.0000    |
| FFM (kg)        | 36.93<br>± 3.97     | 50.2<br>± 6.2       | 0.0000    | 28.63<br>± 3.68    | 38.3<br>± 4.4      | 0.0000    |
| REE (Kcal /min) | 1022.79<br>± 314.52 | 1435.41<br>± 119.62 | 0.0648    | 848.06<br>± 378.69 | 1222.49<br>± 90.91 | 0.0787    |
| TEE (Kcal /min) | 1812.27<br>± 334.8  | 2200.96<br>± 334.93 | 0.003     | 1484.7<br>± 480.96 | 2203.4<br>± 354.07 | 0.0004    |

Table 4 shows comparison of body composition and energy expenditure of Myanmar elderly male and female with those of elderly male and female from the United Kingdom [2,6]. Here also, Myanmar

elderly males and females had lower body weight, lower fat free mass, lower REE and TEE compared with those of elderly males and females from the UK.

## DISCUSSION

In the present study, both REE and TEE of elderly people were lower than those of adults as well as those of Caucasian elderly people. In other words, energy requirement of Myanmar elderly people from Hnin-sigone home for the aged was low.

Several factors contribute to the amount of energy requirement (energy expenditure) by an individual: basal metabolic rate (BMR), physical activity and to a lesser extent diet-induced thermogenesis (DIT). The basal metabolic needs are the energy requirement at complete rest and these are closely related to body composition. The energy required above the basal needs is determined by the level of physical activity. The DIT is the rise in metabolic rate as a consequence of eating and it accounts for about 10% of the energy intake. In general, energy expenditure decreases with increasing age, first because of a decrease in BMR [7].

Schock *et. al.* (1963) attributed a reduction in fat free mass (FFM) with ageing is one of the contributing factors of low BMR in elderly [8]. In the present study, it was also found that FFM of the elderly people was lesser than that of the adults and even among the study groups; the older the age, the lesser was the FFM. In addition, low energy expenditure in elderly is the consequence of a reduction in intensity of physical activity or time spent on physical activity or both [9]. In the present study, reduction in intensity of physical activity as well as time spent on physical activity was observed. Since this study was conducted on institutionalized elderly people, their activities were dictated by the institutional schedule: five hours of the day for meditation, seven and half hours for sleeping, two hours for eating and nine and half hours for leisure. Most of the subjects

spent their leisure time talking, sitting, lying and sleeping rather than walking from place to place. Therefore, half of the day was occupied with lying /sleeping and one third of the day sitting. Only very few persons were very active in that they fully occupied themselves with doing something or walking about.

In the present study, lower resting energy expenditure as well as lesser physical activity could be responsible for lower total energy expenditure.

In addition, there were many controversies over the current recommended daily requirement (RDA) for elderly by FAO/WHO/UNU [10]. That was 1.5 times the BMR. A study with doubly-labelled water (DLW) method in a group of fifteen elderly males of mean age  $69 \pm 19$  years in USA suggested that TEE (1.75 times BMR) may be significantly higher than RDA [6]. In another study of ten elderly females (aged  $73 \pm 3$  years) from UK, TEE was estimated to be a mean of 1.8 times BMR [2]. In the present study, even though REE and TEE were low, the findings were in agreement with those reports that TEE of elderly male was 1.77 times REE and that of elderly female was 1.75 times REE. Therefore, the current RDA recommended by FAO/WHO/UNU for elderly might be lower than what it is supposed to be. This study indicated that the energy requirement of the elderly people from Hninsigone home for the aged was low. The WHO's recommended daily allowance for elderly of 1.5 times the REE might have been underestimated.

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### Nutritive values of popular snacks

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The present study aimed to identify and to determine the nutritive values of the commonly consumed snacks among the students and to explore the factors determining the consumption of varieties of snacks. Identification of currently popular snacks among the students was carried out on a total of 1207 University students of Yangon from January 2001 to December 2001. Both qualitative (focus group discussions) and quantitative methods (structured questionnaire) were employed. Sixteen items each of popular snacks and traditional Myanmar snacks were identified and were analyzed chemically for the nutritive values. The results were compared between the nutritive values of popular snacks and traditional Myanmar snacks. For the same weight of 100 g of snack items, on average, it was found that, higher amount of total calories; protein, carbohydrate and fat were obtained from consumption of popular snacks than from eating traditional Myanmar snacks. But if nutritive values were compared between the two groups for the cost of K.100, eating traditional food would gain more calories, protein, fat, and carbohydrate than the popular snacks. Taste and preference, price, and advertisement were the most common factors to be considered in buying snack foods among the students. Nearly three quarters of the students studied (74.5%) preferred traditional Myanmar foods, being spicy and sour in taste, to the westernized foods. The rest 25.5% of students preferred to take popular snacks thinking that western foods were more hygienic and were attractively packed.

### INTRODUCTION

“Snacks” literally mean hurriedly eaten foods. They are those foods commonly purchased for eating outside the normal pattern of meals. Children, adolescents and young adults are fond of taking snacks. Many factors such as: having enough pocket money, enjoying to be with friends at snack shops and staying more time away from home, development of newer technologies for creating foods with better tastes and textures, media advertisement, development of large scale manufacturing, enabling low cost etc favor the consumption of snacks [1].

Nowadays in Myanmar, fast food centers,

more of westernized style, are sprouted all over the city, Yangon and various attractively packed snack foods, both local-made and imported, can be seen on the shelves of almost every food stores and supermarkets. Researchers are under the impression that these westernized foods and packed snack foods are frequently and fondly taken by young people. Most of the locally made packed snacks have no nutrition facts and labels. Nutritive values of fast foods are not known also.

The prices of these foods are not cheap and thus could lead to economic burden to parents, community and the nation as a whole. From various studies, it was found that 20% to 30% of daily caloric

requirement can be obtained by consumption of snacks in the adolescents [2,3,4]. Therefore, it is important to know the nutritive values of snacks, consumed by this age group. Thus, the present study aimed to identify and determine the nutritive values of the commonly consumed snacks (both traditional and popular snacks) among the students and to explore the factors determining the consumption of varieties of snacks.

## MATERIALS AND METHODS

Identification of currently popular snacks among the students was carried out on a total of 1207 University students of the Institute of Medicine [1], Yangon. Yangon is purposely chosen for being the capital city of Myanmar. Among the Universities/Institutes in Yangon, one University/Institute was randomly chosen. Students from the First Year (entry point) were selected. Food and beverage consumption behavior was qualitatively explored by the Focus Group Discussions (FGDs) and free listing; based upon which the structured questionnaire was constructed for quantification. Seven themes were set for focus group discussions: types of food preferred, perception on having popular snacks among students, influencing factors on consuming popular snacks, coping mechanisms on insufficient pocket money, factors considering on buying snacks, habit of food avoidance, and knowledge on health and nutrition. The guidelines were pre-tested. Some modifications in the way the questions were phrased were made over the course of discussions on the experience from prior discussions. Different items of snacks consumed were asked to list freely also.

Based on the findings of FGDs and free listing, structured questionnaire was constructed and pre-tested. After the explanation by principal investigator, questionnaire forms were self-administered. Among the First Year Students, 1207 (603

males and 604 females), randomly selected were included. Type of snacks consumed, frequency of consumption, places where snacks were usually bought, considering factors on buying of snacks, perception on having advertised snacks, and amount of monthly pocket money or expense etc were included in the questionnaire forms.

The identified snacks were bought from the five randomly chosen snack shops. Five numbers of each item of snacks were collected and sent to the laboratory at Nutrition Research Division, Department of Medical Research (Lower Myanmar) for nutrient analysis. Determination of total protein was done by the estimation of nitrogen by Kjeldahl method [3]. Fat content was estimated by crude ether extraction of dry sample [3]. The content of available carbohydrate was determined by difference, which is by subtracting from 100 to the sum of the values (per 100 g for protein, fat, moisture, sash, and crude fiber) [3]. Then, the nutritive values of these snacks and those of traditional Myanmar snacks were compared.

## RESULTS

Table 1 shows the list of the popular snacks and frequency of intake by the students during the past week. For breakfast, Myanmar traditional snack foods were consumed more frequently than popular snacks. Among the traditional Myanmar snacks, *Pe Pyoke Htamin Kyaw* (fried rice with boiled beans) was taken with the highest frequencies. For the mid-day snacks, both traditional Myanmar snacks and popular snacks were consumed in comparable frequencies. *La Phet Thoke* (Pickled tea leave salad), *Nga Moh Kyaw* and *Ve Ve Cookies* were the commonest items taken as mid-day snacks.

Table 2 shows the nutritive values of different items of traditional Myanmar and popular snacks per 100 gm of weight. Among the traditional snacks *Ba Yar Kyaw* (pounded pea fritter) is most energy dense.

Table 1. Identified commonly consumed traditional Myanmar snacks and popular snacks and percent of respondents consumed

| Traditional Myanmar snacks  | Percent of respondents consumed | Popular snacks                | Percent of respondents consumed |
|-----------------------------|---------------------------------|-------------------------------|---------------------------------|
| <b>Snacks as breakfast</b>  |                                 |                               |                                 |
| <i>Pe Pyoke Htamin Kyaw</i> | 22.9                            | <i>Instant Noodle</i>         | 4.3                             |
| <i>Moh Hin Gah</i>          | 18.5                            | <i>Instant Vermicelli</i>     | 3.4                             |
| <i>Moh Sein Paund</i>       | 12.3                            | <i>Coffee Mix</i>             | 1.7                             |
| <i>Ei Kyar Kwey</i>         | 7.9                             | <i>Cereal Mix</i>             | 1.6                             |
| <i>Platter (Pe/ Thayar)</i> | 5.3                             | <i>Sandwiches</i>             | 1.6                             |
| <i>Samusa</i>               | 2.1                             | <i>Butter cake</i>            | 1.2                             |
| <i>Nan Pya</i>              | 2.1                             | <i>Cake</i>                   | 0.5                             |
| <i>Pe Pyoke</i>             | 2.1                             |                               |                                 |
| <i>Khauk Hnyin Paund</i>    | 1.9                             |                               |                                 |
| <i>Ohn No</i>               | 1.9                             |                               |                                 |
| <i>Khauk Swe</i>            |                                 |                               |                                 |
| <i>Ah Thoke</i>             | 1.7                             |                               |                                 |
| <i>Bain Moh</i>             | 1.6                             |                               |                                 |
| <i>Pauk Sie</i>             | 1.5                             |                               |                                 |
| <i>Ba Yar Kyaw /</i>        | 1.5                             |                               |                                 |
| <i>Nga Pyaw Kyaw</i>        |                                 |                               |                                 |
| <i>Bread and Butter</i>     | 1.3                             |                               |                                 |
| <i>Coffee</i>               | 0.9                             |                               |                                 |
| <i>Tea</i>                  | 0.9                             |                               |                                 |
| <i>Shan Khauk Swe</i>       | 0.7                             |                               |                                 |
| <i>Shwe Htamin</i>          | 0.2                             |                               |                                 |
| <i>San Pyoke</i>            | 0.1                             |                               |                                 |
| <b>Mid-day Sancks</b>       |                                 |                               |                                 |
| <i>La Phet Thoke</i>        | 13.2                            | <i>Ve Ve Cookies</i>          | 10.4                            |
| <i>Nga Moh Kyaw</i>         | 11.6                            | <i>Prawn Crackers</i>         | 9.2                             |
| <i>Ah Lu Kyaw</i>           | 6.2                             | <i>Yummy Flakes</i>           | 8.1                             |
| <i>Coffee</i>               | 5.8                             | <i>Coffee Mix</i>             | 5.1                             |
| <i>Tea</i>                  | 5.2                             | <i>Soft Drinks</i>            | 4.9                             |
| <i>Milk</i>                 | 3.6                             | <i>Donut</i>                  | 2.5                             |
| <i>Tofu Thoke</i>           | 2.1                             | <i>Hamburger</i>              | 2.3                             |
| <i>Yakhine Moh Tee</i>      | 2.0                             | <i>Fired Chicken</i>          | 2.3                             |
| <i>Sprin Roll</i>           | 1.3                             | <i>Pizza</i>                  | 2.1                             |
| <i>Biscuits</i>             | 1.0                             | <i>Chocolate</i>              | 0.7                             |
| <i>Moh Hnin Gah</i>         | 1.0                             | <i>Pie</i>                    | 0.7                             |
| <i>Bain Moh</i>             | 0.9                             | <i>PotatoChips (Imported)</i> | 0.3                             |
| <i>Khauk Moh</i>            | 0.9                             | <i>Hot Dog</i>                | 0.2                             |
| <i>Ah Chin Thoke</i>        | 0.8                             | <i>Barbecue</i>               | 0.1                             |
| <i>Nay Kyar Seed</i>        | 0.6                             | <i>Cream roll</i>             | 0.1                             |

*Nag Pyaw Kyaw* (banana fritter) has the lowest protein content and *La Phet Thoke* has the highest protein content. For the fat content, *La Phet Thoke* contains the highest amount whereas *Kauk Hnyin Pound* (Steamed glutinous rice) contains the lowest amount. Carbohydrate content present is highest in *Ei Kyer Kwey* (fried dough), and lowest in *Ohn No Khauk Swe* (coconut

Table 2. Nutritive values of different items of traditional Myanmar snacks and popular snacks per 100 gm

| Food Items                      | Energy (Kcals) | Protein (gm) | Fat (gm) | Carbohydrate (gm) |
|---------------------------------|----------------|--------------|----------|-------------------|
| <i>Pe Pyoke</i>                 | 193.9          | 4.4          | 3.5      | 36.2              |
| <i>Htamin Kyaw</i>              | 156.0          | 7.2          | 8.1      | 14.5              |
| <i>Moh Hin Gah</i>              | 353.3          | 40.0         | 2.1      | 43.6              |
| <i>Moh Sein Pound</i>           | 478.5          | 18.9         | 36.7     | 27.1              |
| <i>La Phet Thoke</i>            | 392.0          | 7.9          | 16.7     | 52.6              |
| <i>Ei Kyar Kwey</i>             | 358            | 8.4          | 19.0     | 48.1              |
| <i>Thakyar Platter</i>          | 251            | 6.7          | 5.9      | 42.8              |
| <i>Pe Platter</i>               | 228.0          | 5.4          | 5.7      | 38.9              |
| <i>Samusa</i>                   | 261.5          | 9.9          | 9.9      | 33.2              |
| <i>Nan Pya</i>                  | 228.5          | 3.2          | 2.1      | 49.2              |
| <i>Bain Moh</i>                 | 156.5          | 4.6          | 6.3      | 20.8              |
| <i>Ah Thoke</i>                 | 225            | 8.0          | 17.2     | 10.7              |
| <i>Ohn No</i>                   |                |              |          |                   |
| <i>Khauk Swe</i>                | 211.4          | 5.0          | 1.0      | 45.6              |
| <i>Kauk Hnyin Pound</i>         | 309            | 7.5          | 19.3     | 68.7              |
| <i>Pauk Sie (Wetter)</i>        | 583            | 11.3         | 38.8     | 47.4              |
| <i>Ba Yar Kyaw</i>              | 266            | 2.5          | 8.0      | 46.0              |
| <i>Nga Pyaw Kyaw</i>            |                |              |          |                   |
| <b>Popular Snacks</b>           |                |              |          |                   |
| <i>Instant Noodle</i>           | 445.6          | 9.6          | 19.1     | 58.8              |
| <i>Butter Cake</i>              | 440.2          | 4.7          | 29.2     | 39.6              |
| <i>Instant Vermicelli</i>       | 420.9          | 6.6          | 6.6      | 83.8              |
| <i>Coffee Mix</i>               | 387.3          | 1.3          | 1.3      | 92.6              |
| <i>VeVe Cookies</i>             | 429.3          | 4.5          | 11.6     | 76.8              |
| <i>Prawn Crackers</i>           | 405.5          | 6.13         | 7.9      | 77.3              |
| <i>Yummy Flakes</i>             | 442.6          | 5.4          | 14.6     | 72.4              |
| <i>Donut</i>                    | 382.8          | 3.4          | 16.2     | 55.9              |
| <i>Sandwiches (Cheese)</i>      | 431.0          | 6.2          | 18.2     | 38.2              |
| <i>Hamburger (Chicken)</i>      | 331.3          | 8.9          | 23.3     | 21.5              |
| <i>Cereal mix</i>               | 405.2          | 4.2          | 2.4      | 91.7              |
| <i>Fried Chicken</i>            | 371.4          | 18.7         | 32.7     | 0.6               |
| <i>Pizza</i>                    | 258.4          | 5.7          | 15.5     | 24.0              |
| <i>Chocolate</i>                | 534.1          | 6.8          | 30.2     | 58.1              |
| <i>Pie</i>                      | 500.0          | 4.3          | 30.4     | 53.2              |
| <i>Potato Crisps (Imported)</i> | 566.4          | 3.5          | 38.9     | 53.1              |

\* Five in numbers of each item of snacks

noodle). Among the popular snacks, potato chips are the most energy dense while chicken pizza is least energy dense. Fried chicken has the highest protein content and coffee mix has the lowest protein content. Fat is present in highest amount in potato chips while present in lowest amount in coffee mix. Highest carbohydrate value can be obtained by taking 100 gm of instant vermicelli.

Table 3 shows the mean calorie, protein, carbohydrate, and fat contents of both types of snacks for 100 *kyats*. The calorie contents of both types of snacks are listed

from the highest to the lowest. In traditional Myanmar snacks, the amount of calories of

highest and that of *Thakyar Platter* is the lowest content for 100 kyats. In comparison

Table 3. Mean caloric, protein, carbohydrate, and fat contents of both types of snacks for 100 Kyats

| Traditional Myanmar snacks        | Energy (Kcals) | Protein (g) | Carbohydrate (g) | Fat (g)      |
|-----------------------------------|----------------|-------------|------------------|--------------|
| <b>Traditional Myanmar Snacks</b> |                |             |                  |              |
| <i>Ba Yar Kyaw</i>                | 2532.0         | 45.2        | 189.6            | 155.2        |
| <i>Moh Sein Pound</i>             | 2356.2         | 40.8        | 444.7            | 29.6         |
| <i>Nga Pyaw Kyaw</i>              | 1995.0         | 18.8        | 345.0            | 60.0         |
| <i>Moh Hin Gah</i>                | 1611.0         | 15.0        | 169.2            | 77.9         |
| <i>Thakyar Platter</i>            | 1449.9         | 15.0        | 194.8            | 77.0         |
| <i>Kauk Hnyin Pound</i>           | 1446.7         | 33.3        | 304.0            | 66.7         |
| <i>Pe Platter</i>                 | 1380.5         | 27.1        | 235.4            | 32.5         |
| <i>Ei Kyar Kwey</i>               | 1128.9         | 22.8        | 151.5            | 48.1         |
| <i>Bain Moh</i>                   | 1108.8         | 39.4        | 236.2            | 10.1         |
| <i>Ah Thoke</i>                   | 1072.8         | 33.1        | 149.8            | 45.4         |
| <i>La Phet Thoke</i>              | 1051.6         | 41.6        | 59.6             | 80.7         |
| <i>Ohn No Khauk Swe</i>           | 1047.6         | 53.4        | 90.2             | 50.7         |
| <i>Pe Pyoke Htamin Kyaw</i>       | 1005.0         | 22.0        | 181.0            | 17.5         |
| <i>Samusa</i>                     | 820.8          | 21.2        | 140.0            | 20.5         |
| <i>Pe Pyoke Nan Pya</i>           | 724.0          | 19.8        | 66.4             | 19.8         |
| <i>Pauk Sie (Wettar)</i>          | 706.1          | 19.3        | 54.7             | 44.4         |
| <b>Mean</b>                       | <b>1358.6</b>  | <b>28.4</b> | <b>191.8</b>     | <b>52.2</b>  |
| <b>Popular Snacks</b>             |                |             |                  |              |
| <i>Instant Vermicelli</i>         | 781.6          | 12.3        | 155.6            | 12.3         |
| <i>Butter Cake</i>                | 657.8          | 7.0         | 59.2             | 43.6         |
| <i>Sandwiches (Cheese)</i>        | 452.6          | 6.5         | 40.1             | 19.1         |
| <i>Coffee Mix</i>                 | 397.8          | 1.3         | 92.6             | 1.3          |
| <i>Fried Chicken</i>              | 371.4          | 18.7        | 0.6              | 32.7         |
| <i>Cereal Mix</i>                 | 351.6          | 3.6         | 79.6             | 2.1          |
| <i>Ve Ve Cookies</i>              | 303.3          | 25.4        | 54.2             | 8.2          |
| <i>Yummy Flakes</i>               | 297.9          | 3.6         | 48.7             | 9.8          |
| <i>Hamburger (Chicken)</i>        | 289.9          | 7.8         | 18.8             | 20.4         |
| <i>Donut</i>                      | 279.2          | 2.5         | 40.8             | 11.8         |
| <i>Prawn Crackers</i>             | 273.7          | 4.1         | 52.2             | 5.3          |
| <i>Pizza (Chicken)</i>            | 242.2          | 5.3         | 22.5             | 14.5         |
| <i>Potato Chisps (Imported)</i>   | 184.1          | 1.1         | 17.3             | 12.3         |
| <i>Chocolate</i>                  | 159.3          | 2.0         | 17.3             | 9.0          |
| <i>Pie (Strawberry)</i>           | 115.0          | 0.9         | 12.3             | 7.0          |
| <b>Mean</b>                       | <b>364.8</b>   | <b>5.95</b> | <b>50.1</b>      | <b>14.9*</b> |

\* Statistically different from mean caloric contents of traditional Myanmar snacks and currently popular snacks ( $p < 0.05$ )

*Moh Sein Pound* is the highest and that of *Pauk Sie* is the lowest content for 100 kyats. In comparison, the calorie contents of currently popular snacks, the instant vermicelli has the highest amount where as the strawberry cream pie contains the lowest amount for 100 kyats. Mean calories contents of them are statistically different ( $p < 0.05$ ). The highest caloric content of traditional Myanmar snacks is three times more than that of currently popular snacks, i.e., 2656.5 Kcals vs 781.6 Kcals. The protein contents of both types of snacks for 100 kyats are listed from the highest to the lowest. In traditional Myanmar snacks, the amount of protein of *Ba Yar Kyaw* is the

of the protein contents of currently popular snacks, the fried chicken has the highest amount where as the strawberry cream pie contains the lowest amount for 100 kyats. Mean protein contents of them are statistically different ( $p < 0.5$ ). The highest protein content of traditional Myanmar snacks is two times more than that of currently popular snacks, i.e., 45.2 gm vs 18.7 gm. Mean fat contents of both types of snacks for 100 kyats are listed from the highest to the lowest. In traditional Myanmar snacks, the amount of protein of *Ba Yar Kyaw* is also the highest and that of *Bain Moh* is the lowest content for 100 kyats. In comparison of the fat contents of

currently popular snacks, the butter cake has the highest amount where as the coffee mix contains the lowest amount for 100 kyats. Mean fat contents of them are significantly different ( $p<0.05$ ). The highest fat content of traditional Myanmar snacks is three times more than that of currently popular snacks, i.e., 155.2 gm vs 43.6 gm. The carbohydrate contents of both types of snacks for 100 kyats are also listed from the highest to the lowest. In traditional Myanmar snacks, the amount of carbohydrate of *Moh Sein Pound* is the highest and that of *Pauk Sie* is the lowest content for 100 kyats. In comparison of the carbohydrate contents of currently popular snacks, chicken pizza has the highest amount where as the fried chicken contains the lowest amount for 100 kyats. Mean carbohydrate contents of them are significantly different ( $p<0.5$ ). The highest carbohydrate content of traditional Myanmar snacks is two times more than that of currently popular snacks, i.e., 444.7 gm vs 242.0 gm.

Table 4 shows the food behavior of First M.B., B.S. students, Institute of Medicine [1]. Most of the students bought the snacks according to their preference. They also considered health benefits of consuming snacks. The next considered factor on buying of snacks was the price. Although, the least considering factor was the advertisement, more than 75% of the students had the habit of eating the advertised foods. The common reason for consumption of advertised foods was to test the taste. If they had liked the taste, they would have consumed the next time. Only 1.2 % of the respondents were influenced by peers in consumption of advertised snacks. Nearly three quarters of respondents (74.5%) preferred Myanmar food to westernized food. Familiar with Myanmar food was the commonest reason for preference. The reasonable price was also one of the factors for preference of traditional Myanmar food. Three percent of respondents thought that traditional Myanmar foods were healthier than

westernized foods. Some of the respondents (15.5%) preferred westernized food to Myanmar food because of their attractive packing. Some students (9.6%) said that westernized food was more hygienic than Myanmar food. Most of the respondents (57.9%) would ask their parents for the money again, if their pocket money was not enough. Some of the respondents (16.8%) reduced in buying new dress or doing some recreational activities. And, few respondents (2.5%) solved that kind of problem by doing part-time jobs (e.g study guide).

Table 4. Food behavior of First M.B.,B.S. students, Institute of Medicine (1)

| Variables                                           | Percent of respondents |
|-----------------------------------------------------|------------------------|
| Factors considering on buying of snacks             |                        |
| Price                                               | 21.7                   |
| Preference                                          | 88.4                   |
| Advertisements                                      | 15.9                   |
| Benefits for nutrition / health                     | 26.6                   |
| Practice on consumption of advertised snacks        |                        |
| Presence                                            | 56.3                   |
| Absence                                             | 43.7                   |
| Reasons for consumption of advertised snacks        |                        |
| Testing the taste                                   | 57.6                   |
| Being preference                                    | 40.0                   |
| Being prestige / Peer influence                     | 10.2                   |
| Winning the lucky draw                              | 1.2                    |
| Food preference                                     |                        |
| Myanmar food                                        | 74.5                   |
| Westernized food                                    | 25.5                   |
| Reasons for preference of Myanmar food              |                        |
| Sharp in taste (spicy, sour and salty)              | 15.0                   |
| Easily prepared by themselves                       | 3.0                    |
| Easily available                                    | 4.0                    |
| Familiar                                            | 17.5                   |
| Reasonable price                                    | 3.0                    |
| More suitable for health                            | 3.0                    |
| Reasons for preference of westernized food          |                        |
| More hygienic than Myanmar food                     | 9.6                    |
| Attractive packing                                  | 15.6                   |
| Easily available                                    | 4.0                    |
| Solutions to the problem of inadequate pocket money |                        |
| Ask for from their parents again                    | 57.9                   |
| Do part-time work                                   | 2.4                    |
| Reduce in buying new dresses / doing entertainment  | 16.8                   |

## DISCUSSION

During the past few years, due to the appearance of food industries, many items of snack foods, both imported and locally produced, have accommodated the shelves of supermarkets, food shops and markets.

Many of these food items are advertised and become popular. Snack foods, which are attractively packed, reach not only to the cities (urban areas) but also to the rural villages. They become popular, readily available, and are frequently consumed by people, especially by the children and adolescents. Although the prices of these snack foods are not cheap in comparison to the traditional Myanmar snacks, children and adolescents like these snacks very much and spend much money on buying them. From this study on the University students, 16 items each of currently popular snacks and traditional Myanmar snacks, most frequently consumed by the students, were identified. For breakfast, traditional Myanmar snacks were consumed more than the popular snacks as traditional snacks were more available in the morning, filled the stomach more and prepared by the parents who preferred low cost. In our country, breakfast is usually taken at homes together with their parents. However, currently popular snacks were consumed much more frequently in mid-day as they were more readily available at the canteens, convenient and less parental influence in the mid-day. From the nutritive values of traditional Myanmar snacks, *Bayar Kyaw* was found to be most energy dense containing 583 kcals. per 100 grams. This is due to the presence of high content of fat and protein as it is fried and made from beans. *Moh Hin Gah* is found to have least energy content for 100 grams. It might be because, *Moh Hin Gah* is a kind of soup containing vermicelli and small amount of fish and other ingredients. The high protein and fat contents of *La Phet Thoke* were due to the presence of varieties of beans, dried prawn and oil on top. *Ei Kyar Kwey* is made up of wheat and then dough so that the carbohydrate content is high.

When compared to the above mentioned traditional Myanmar snacks, currently popular snacks contained more energy, fat and carbohydrate and less protein for 100 g of foodstuff. These foods are energy dense but not nutrient dense. Frequent consump-

tion of these kinds of foods can lead to obesity. When comparison of the nutritive values of traditional Myanmar snacks and currently popular snacks for the cost of K. 100 was made, it was found that consuming traditional Myanmar snacks would obtain more calories, protein, fat, and carbohydrate than eating popular snacks as the latter foodstuffs are more expensive.

Thus, from the findings of this study, we can conclude that consuming traditional Myanmar snacks is more nutritious and cheaper than eating popular snacks and Myanmar populace, especially children and adolescents should be given proper information to be able to choose healthier foods with less costs.

To reach the University education level, parents must be able to support and afford. That is why, most of them consider taste and preference in buying the snack foods rather than the price, although price is one of the factors considered also. Similar findings were seen in the study of Shannon *et. al.* (2002) [5] on the high school students, where taste and getting a lot for their money were the major influencing factors on their food choices. This finding is in contrast to that found when we studied on the housewives, who considered mostly price in buying foods [6]. Young people would like to try new experiences, thus more than 50% of the respondents tried advertised foods to know the taste. For the housewives of our country, most of them did not buy advertised foods as they said, for the same quality, advertised foods were more expensive. Being the University students, they also considered health and nutrition aspects when buying snacks.

Food industries captured the fact that children and adolescents considered taste and preference most in buying snacks and produced food items that would taste better and attractively packed. But these kinds of foods are not healthy: rich in energy and fat, lack other nutrients (protein, vitamins, and minerals). Besides, they are more costly

than traditional Myanmar snacks. These findings suggest that nutrition education, proper food labeling, improving the taste of low-fat and nutrient dense food may offer key strategies for promoting healthy food choices among the students.

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## ***In vitro* antimicrobial activity of *Lawsonia alba* (Dan-gyi)**

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Water soluble crude extract of *Lawsonia alba* (Dan-gyi) was tested against two isolates of *Entamoeba histolytica* and fourteen strains of pathogenic bacteria. The growth patterns of amoebae in cultures were determined at time intervals of 12 and 24 hours respectively after exposure to the diluted extract. The *in vitro* bactericidal and bacteriostatic activities of *L. alba* extract were also tested by agar disc diffusion technique and by serial tube dilution technique. The Minimum Amoebicidal Concentration (MAC) of the crude extract of *L. alba* was found to be 100µg/ml. The extract of *L. alba* was found to possess antibacterial activity on some pathogenic bacteria. Out of fourteen strains of bacteria tested, six strains were inhibited at the concentration of 0.5 mg/ml and two strains were inhibited at 1mg/ml concentrations.

## **INTRODUCTION**

*Lawsonia alba* (Syn: *Lawsonia inermis*; common name Henna, Mehndi, Mendika; Myanmar name: Dan-gyi) is a perennial shrub which grows widely in Myanmar. Henna is the Persian name and is native to Asia and Mediterranean coast of Africa and now thrives in warmer climates all over the world. The history and origin of Dan-gyi (Henna) are hard to track, with centuries of migration and cultural interaction, it is difficult to determine where particular traditions began. There are some historic evidences that Dan-gyi originated in ancient India. Others believe that Dan-gyi was introduced to India in the 12<sup>th</sup> century. This plant has been used for at least 5,000 years as a cosmetic and for its natural healing properties. There is also a documentation that Henna was used in ancient Egypt to stain fingers and toes of the Pharaohs prior to mummification. Dan-gyi is also used as a medicinal plant because of its attributed antibacterial, antifungal, astringent, anti-hemorrhagic, hypotensive and sedative effects. It has also been used as a folk

remedy against headache, jaundice and leprosy in many world cultures [1, 2].

In this present study, a water soluble extract was prepared from mature leaves of Dan-gyi and its *in vitro* activities on two isolates of *Entamoeba histolytica* and fourteen strains of bacteria were tested.

## **MATERIALS AND METHODS**

### *Extraction of leaves*

Air dried leaves (370 gm) were refluxed for two hours in two liters of petroleum ether and filtered. The leaves residue were dried in air and extracted with two liters of 50% ethanol and filtered. Fifty percent alcoholic solution was concentrated under reduced pressure at low temperature. This procedure was carried out in Soxlet apparatus after removal of chlorophyll and waxy substances according to the method of Hanke and Talaat (1961) [3]. Fractionations were made to remove pectate after treatment with calcium hydroxide. The total extract weight obtained was approximately 40 gm. The

alcoholic substance was again eluted with two liters of distilled water and concentrated under reduced pressure. Weight obtained after concentration was approximately 30 g. This water soluble extract was used throughout the experiment. The extract was initially dissolved in sterile double distilled water and the pH was adjusted between 6.8 to 7.2. The desired concentrations were obtained by dilution.

#### *In vitro amoebicidal activity*

Stool samples containing active trophozoites of *Entamoeba histolytica* were collected from dysenteric patients attending Infectious Diseases Hospital, North Okkalapa, Yangon. Out of five samples collected, only two samples showed satisfactory growth and they were isolated to be free from other symbionts such as *Blastocystis hominis*. These two samples were designated as DMR/Eh 005 and DMR/Eh 006 and cultured continuously in two biphasic media [4,5]. Amoebicidal activity of the extract of *L. alba* was assayed by the method of Brackett and Blenick, 1947 [6] with minor modifications by Myint Oo and co-workers, 1972 [7]. Emetine hydrochloride (Biochemical Research, USA) and metronidazole (Myanmar Pharmaceutical Factory, Yangon) were used as control drugs. Before each experiment, the test and control cultures were ascertained to contain approximately equal numbers of trophozoites. Ten replicate countings of amoeba were made at 12 and 24 hours intervals according to the method described by Myint Oo 1989 [8].

#### *Screening of in vitro antibacterial activity*

Screening of antibacterial activity was done by the use of impregnated filter-paper discs. The discs, 8 mm in diameter, and punched from filter papers of Whatman Grade 17 was sterilized by autoclaving followed by drying in an oven. These were then impregnated with diluted extracts and fractions of *L. alba* extract (A & B) and then allowed to dry at room temperature

under sterile condition. Test organisms used were *Escherichia coli* (O127/B2, WR2/69), *Klebsiella pneumoniae* (DMR), *Proteus mirabilis* (IM1), *P. vulgaris* (IM1), *Pseudomonas fluorescens* (WR2/69), *P. aeruginosa* (W12/69), *Salmonella typhi* (ATCC 992V), *Shigella boydii* (NHL), *S. dysenteriae* (NHL), *Shigella shigae* (NHL), *Staphylococcus aureus* (WS/69), *S. epi-dermidis* (WR1/69) and *Vibrio cholerae* ElTor (IM1). A few colonies of the pure cultures were inoculated into 2–4 ml of Trypticase soy broth. These tubes were incubated for 2–4 hours in a waterbath at 37°C to produce a bacterial suspension with barely visible to moderate cloudiness. This contained approximately  $10^5$  to  $10^7$  organisms per milliliter of suspension. The organisms were then streaked onto the surface of Oxoid Sensitivity test agar with a swab. After the inoculum had dried, the dried discs were placed on the medium. A control disc (no drug), impregnated with solvent only and known standard control disc of tetracycline and ampicillin were also placed onto the medium. After overnight incubation at 37°C, the zones of inhibition were measured. The bactericidal or bacteriostatic action and the minimum inhibitory concentration were also determined by serial tube dilution method. Comparison of three media namely Oxoid Sensitivity Test agar, Trypticase agar and Nutrient agar were also determined.

### RESULTS

Table 1 shows the *in vitro* amoebicidal concentrations of emetine, metronidazole and water soluble extracts of *L. alba*. Two strains of *E. histolytica* grown in two types of media were used for comparative study. The minimum amoebicidal concentrations were not significantly different between variation of strains and culture medium in this study. The mean growth rates of two strains of *E. histolytica* after exposure to extracts of *L. alba* (100µg/ml), metronidazole (20µg/ml) and emetine hydrochloride (20µg/ml) are shown. Each drug was added after 24 hours of cultivation in two types of

media. A significant inhibition of water soluble extract of *L. alba* was observed at the concentration of 100µg/ml.

Table 2 shows the mean diameters of zone of inhibition by *L. alba* extract. Only *Pseudomonas fluorescens* was not inhibited by the extract of *L. alba*.

Table 1. Minimum amoebicidal concentrations of *L. alba* extract and control drugs on two isolates of *Entamoeba histolytica* grown in two different media *in vitro*

| Isolates of <i>E. histolytica</i> | Types of Medium         | Minimum Amoebicidal Concentration (µg/ml) |               |                        |
|-----------------------------------|-------------------------|-------------------------------------------|---------------|------------------------|
|                                   |                         | Emetine HCl                               | Metronidazole | <i>L. alba</i> extract |
| DMR/ Eh 005                       | Dobell & Laidlaw (1926) | 25                                        | 50            | 150                    |
|                                   | Rao (1951)              | 25                                        | 25            | 100                    |
| DMR/ Eh 006                       | Dobell & Laidlaw (1926) | 25                                        | 50            | 150                    |
|                                   | Rao (1951)              | 25                                        | 50            | 150                    |

Table 3 represents the Minimum Inhibitory Concentrations (MICs) of *L. alba* extract on some pathogenic bacteria. It was observed that the extract of *L. alba* inhibits the growth of *Proteus mirabilis*, *Proteus vulgaris*, *Shigella flexneri*, *Staphylococcus aureus* and *Vibrio cholerae* ElTor at the concentration of 500µg/ml, whereas *Escherichia coli*, *Shigella boydii*, and *Shigella schmitzi* were inhibited only at the concentrations of 1 mg/ml. Table 4 represents the comparison of zones of inhibition in three different medias. Oxoid Sensitivity Test agar (OSTA) and Trypticase Soy agar (TSA) showed no significant variation but the inhibition zones were markedly smaller in some bacteria grown on Nutrient agar (NA).

## DISCUSSION

In 1961, Hanke and Talaat [3] studied the biochemistry and physiology of *L. alba*. They had also conducted a clinical

Table 2. *In vitro* bactericidal activity of *Lawsonia alba* extract measured by paper disc method

| Sr No | Organisms                           | Diameter of zone of inhibition (mm) |            |            |               |              |
|-------|-------------------------------------|-------------------------------------|------------|------------|---------------|--------------|
|       |                                     | <i>Lawsonia alba</i> extract        |            |            | Control drugs |              |
|       |                                     | Crude                               | Fraction A | Fraction B | Ampicillin    | Tetracycline |
| 1     | <i>Escherichia coli</i> O127/ B8;   | 11                                  | NT         | NT         | 0             | 16           |
| 2     | <i>Klebsiella pneumoniae</i>        | 12                                  | NT         | NT         | 15            | 15           |
| 3     | <i>Proteus mirabilis</i>            | 22                                  | 17         | 17         | 20            | 10           |
| 4     | <i>Proteus vulgaris</i>             | 21                                  | 11         | 18         | 14            | 10           |
| 5     | <i>Pseudomonas fluorescens</i>      | 0                                   | 12         | 23         | NT            | NT           |
| 6     | <i>Salmonella typhi</i>             | 14                                  | 10         | 10         | 20            | 24           |
| 7     | <i>Shigella boydii</i>              | 16                                  | NT         | NT         | 20            | 16           |
| 8     | <i>Shigella dysenteriae</i>         | 21                                  | NT         | NT         | 30            | 14           |
| 9     | <i>Shigella flexneri</i>            | 12                                  | NT         | NT         | 30            | 13           |
| 10    | <i>Shigella schmitzi</i>            | NT                                  | 12         | 10         | 28            | 14           |
| 11    | <i>Staphylococcus aureus aureus</i> | 24                                  | NT         | NT         | 14            | 14           |
| 12    | <i>Vibrio cholerae</i> ElTor        | 15                                  | 12         | 10         | 24            | 30           |

NT=Not tested

trial in. intestinal amoebiasis. The dried powder of leaves, given to the patients suffering from amoebic dysentery was found to be effective and no sign of toxicity was observed. As far as the authors are aware, there is no published report on *in vitro* amoebicidal activity of the extract of *L. alba*. However, it could be a useful herbal agent in clinical practice after further purification and a series of *in vivo* testings.

In 1968, Malekzadeh *et. al.* [9] used aqueous extract to search for its inhibitory effect on eight species of pathogenic bacteria (*Bacillus cereus*, *Bacillus anthracis*, *Escherichia coli*, *Proteus vulgaris*, *Agrobacterium tumefaciens*, *Santhomius campestris*, *Staphylococcus aureus* and *Erwinia carotoroa*). This aqueous extract was active against all eight species of bacteria at the concentration of

100µg/ml. Out of eight species of bacteria tested by Malekzadeh [9], three different strains of bacteria were also included in our study.

Table 3. Minimum Inhibitory Concentrations of *Lawsonia alba* extract on some pathogenic bacteria

| Sr. No. | Tested bacteria                             | Minimum Inhibitory Concentration (mg/ml) |
|---------|---------------------------------------------|------------------------------------------|
| 1       | <i>Escherichia coli</i> O127/B8; WR2/69     | 1.0                                      |
| 2       | <i>Klebsiella pneumoniae</i> , DMR          | NT                                       |
| 3       | <i>Proteus mirabilis</i> , IM1              | 0.5                                      |
| 4       | <i>Proteus vulgaris</i> , IM1               | 0.5                                      |
| 5       | <i>Pseudomonas fluorescens</i> , WR2/69     | NT                                       |
| 6       | <i>Salmonella typhi</i> , ATCC 992V         | NT                                       |
| 7       | <i>Shigella boydii</i> , NHL                | 1.0                                      |
| 8       | <i>Shigella dysenteriae</i> , NHL           | NT                                       |
| 9       | <i>Shigella flexneri</i> , NHL              | 0.5                                      |
| 10      | <i>Shigella schmitzi</i> , NHL              | 1.0                                      |
| 11      | <i>Staphylococcus aureus aureus</i> , WS/69 | 0.5                                      |
| 12      | <i>Vibrio cholerae</i> EITor, IM1           | 0.5                                      |

NT=Not tested

Although the extraction method was not the same, extract of *L. alba* showed marked inhibition on these three bacteria (*E. coli*, *P. vulgaris* & *S. aureus*) used in our study. It has also been proven in a study in Myanmar that *L. alba* extract exerted the acceleration of wound healing activity in *in vivo* experimentally induced wound infections in rats [10]. From this study, extract of *L. alba* showed promising results of *in vitro* antimicrobial activity on *E. histolytica* and some pathogenic bacteria. Thus, further purification and investigation should be carried out to obtain a potent antimicrobial herbal agent from *L. alba*. The active substance of the plant is believed to be "lawsone" (2-hydroxy-1, 4 - naphthoquinone) which could be isolated from leaves and other parts of the plant.

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Table 4. Comparison of growth of bacterial species on three different media

| Sr No. | Tested bacteria                             | Diameters of zones of inhibition (mm) |     |    |
|--------|---------------------------------------------|---------------------------------------|-----|----|
|        |                                             | OSTA                                  | TSA | NA |
| 1      | <i>Escherichia coli</i> O127/B8; WR2/69     | 22                                    | 23  | 18 |
| 2      | <i>Klebsiella pneumoniae</i> , DMR          | NT                                    | NT  | NT |
| 3      | <i>Proteus mirabilis</i> , IM1              | 22                                    | 24  | 18 |
| 4      | <i>Proteus vulgaris</i> , IM1               | 28                                    | 30  | 21 |
| 5      | <i>Pseudomonas fluorescens</i> , WR2/69     | NT                                    | NT  | NT |
| 6      | <i>Salmonella typhi</i> , ATCC 992V         | 16                                    | 14  | 12 |
| 7      | <i>Shigella boydii</i> , NHL                | 20                                    | NT  | 16 |
| 8      | <i>Shigella dysenteriae</i> , NHL           | NT                                    | NT  | NT |
| 9      | <i>Shigella flexneri</i> , NHL              | NT                                    | 27  | 21 |
| 10     | <i>Shigella schmitzi</i> , NHL              | NT                                    | 24  | 23 |
| 11     | <i>Staphylococcus aureus aureus</i> , WS/69 | NT                                    | 19  | 16 |
| 12     | <i>Vibrio cholerae</i> EITor, IM1           | 15                                    | 15  | 12 |

OSTA = Oxoid Sensitivity Test Agar

TSA = Trypticase Soy Agar

NA = Nutrient Agar

NT = Not tested

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**Effect of pomegranate juice supplementation on  
serum total cholesterol level of hypercholesterolemic rats**

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To investigate the effect of pomegranate (*Punica granatum*) juice on serum total cholesterol level in an animal model, an experimental study was carried out in twenty Wistar strain rats (10 males and 10 females, 200-250 gm body weight.). To induce hypercholesterolemia, all rats were supplemented with a high cholesterol diet which was enriched with coconut oil, cholesterol and sugar (25%, 1%, and 16% by weight, respectively) to the ordinary feed for thirty days. After dietary inducement, ten of twenty hypercholesterolemic rats whose serum total cholesterol levels increased by 75% and above 75% than their basal levels were administered orally, an alcohol extract of pomegranate seeds (0.056 gm) and 3 ml of fresh pomegranate juice (PJ) daily for ninety days. Another ten hypercholesterolemic rats (control group) were administered orally, 3 ml of distilled water daily for ninety days. Serum total cholesterol levels were measured on Day 30, Day 60, and Day 90 of pomegranate juice and distilled water supplementation. Serum total cholesterol levels of the test group were lower than those of the control group on Day 30 ( $91.44 \pm 22.9$  mg/dl vs  $109.4 \pm 27.19$  mg/dl), and on Day 60 ( $82.6 \pm 17.3$  mg/dl vs  $89.7 \pm 13.96$  mg/dl) of supplementation, although they were not statistically significant ( $p > 0.05$  for both). On Day 90 of supplementation, serum total cholesterol level of test group was significantly lower than that of control group ( $74.4 \pm 10.11$  mg/dl vs  $86.8 \pm 10.52$  mg/dl), ( $p = 0.043$ ). Therefore, a 90-day supplementation of PJ reduces serum total cholesterol level in diet-induced hypercholesterolemic rats.

## INTRODUCTION

Hypercholesterolemia in humans is considered to be elevated blood cholesterol level (ie. 240 mg% and above). Hypercholesterolemia is usually discovered during routine screening and is an asymptomatic condition. It is more common in individuals with family history of it, but life style factors (eg. diet in saturated fat) clearly play major role. Having too much cholesterol in blood is not a disease itself but can lead to the hardening and narrowing of the arteries (atherosclerosis) in the major vascular systems. Therefore, it is associated with myocardia infarction, cerebrovascular accident and intermittent claudication.

At early stage of atherosclerosis, macrophage cholesterol (and oxidized lipid)

accumulation and foam cell formation take place, leading to the development of the complicated atherosclerotic lesion [1]. Major contributors to cholesterol accumulation in arterial cells during atherogenesis include high plasma cholesterol concentration [2], increased oxidative stress [3], reduced serum paraoxonase activity [4], increased uptake of atherogenic lipoproteins by arterial cells [5], enhanced macrophage cholesterol esterification rate [6], and decreased cholesterol efflux from arterial cells [7]. Since oxidative stress is believed to play an important role in early atherogenesis, antioxidants (polyphenolic flavonoids) which are rich in red wine, grapes, licorice, ginger and pomegranate, significantly reduce oxidative stress by inhibiting the formation of OX-LDL and macrophage lipid peroxidation.

Pomegranate (*Punica granatum* L, *Punicaceae*) or *Thalathee* (ovJoD:), likely originated in Iran and Afghanistan, is currently grown mainly in Iran, India, and the United States, but also in most near and far east countries [8]. Historically, the fruit is mentioned by various cultures and religions. Greek and Persian mythologies mention the fruit as representing life, regeneration, and marriage. The ancient Chinese believed that the seeds symbolized longevity and immortality. The fruit is also a symbol of resurrection and life in Christianity, and it is one of the three “blessed fruits” in Buddhism [9].

Aviram *et. al.* [10] showed that PJ supplementation of apolipoprotein E deficient ( $E^0$ ) mice reduced the size of their atherosclerotic lesions by 44%. It was also found that PJ consumption for 3 years by patients with carotid artery stenosis reduced common carotid intima-media thickness, blood pressure and LDL oxidation [11]. Therefore, the effect of pomegranate grown in Myanmar on plasma lipids profile has to be investigated. The objective of this study was to investigate the effect of pomegranate (*Punica granatum*) juice on serum total cholesterol level of rats with a high cholesterol diet prior to pomegranate juice supplementation.

## MATERIALS AND METHODS

An experimental study design with controlled trial was used in twenty Wistar strain rats (200-250 gm body weight, 10 males and 10 females). To induce hypercholesterolemia, all rats were fed with a high cholesterol diet for thirty days before pomegranate juice (PJ) supplementation. After dietary inducement, the rats whose serum total cholesterol levels were increased by 75% and above 75% than their basal levels were defined as hypercholesterolemic rats and they were allocated into two groups (Test and Control Group). Then, the high cholesterol diet was changed into the routine diet on the first day of PJ

supplementation and continued throughout the study. All rats were housed individually in metabolic cages for three days to measure food intake during dietary inducement, first, second and third month of PJ supplementation. Body weights and serum total cholesterol levels were determined before and after dietary inducement and Day 30, Day 60, and Day 90 of PJ supplementation.

### *Dietary inducement for hypercholesterolemia*

All rats were maintained at 24 C° with a 12-hour light and dark cycle. Initial body weights were measured with an animal balance and basal serum total cholesterol levels of them were determined. To make a high cholesterol diet, a routine diet was enriched with coconut oil, sugar, and cholesterol in the ratio of 25%, 16%, and 1% by weight of feed respectively. Then, all rats were given freely access to high cholesterol diet for thirty days. After that, serum total cholesterol levels were measured and by an operational definition, the hypercholesterolemic rats were undergone the intervention study. If the rats whose serum total cholesterol levels increased by less than 75% than their basal levels, they were continued to feed the high cholesterol diet for next thirty days.

### *Pomegranate Juice (PJ) Preparation*

Pomegranates were provided by Ministry of Agriculture and Irrigation, in February, 2005. After peeling off the covers, the red juicy edible sacs together with seeds were crushed with a blender. The pink fluid and crushed seeds were separated by filtration and pink fluid was kept as 15 ml aliquots and was stored at -80C°. The crushed seeds were kept to be dry under shadow and extracted with 50% ethyl alcohol. The dried alcohol extract of the crushed seeds was stored separately and immediately before administration to rats, pink fluid and alcohol extract of crushed seeds were mixed to make pomegranate juice for rats.

### Pomegranate Juice (PJ) Supplementation

After dietary inducement, the high cholesterol diet was changed to the routine diet and hypercholesterolemic rats were allocated into two groups: 10 in test group and 10 in sex-matched control group. If a person ate a pomegranate a day, a rat would have 0.5ml fresh juice containing 0.00028g of alcohol extract of seeds because of the differences between their body weights. To evaluate pharmacological effects of herbs, the dose of PJ for administration to rats was increased up to six times of human dose. Therefore, it became 3 ml of pomegranate pink fluid containing 0.056 gm of alcohol extract of pomegranate seeds. Test and control groups were administered orally pomegranate juice and three ml of distilled water, respectively, for ninety days. Either a high cholesterol diet or routine diet, all rats were fed without restriction till the end of the study. Before PJ supplementation, qualitative phyto-chemical analysis of seeds and fluid was carried out in Laboratory of Pharmacology Research Division [12].

Blood samples were collected from tails to determine serum total cholesterol levels on Day 30, Day 60, and Day 90. Serum total cholesterol levels were determined spectrophotometrically with commercially available reagent kits (Hospitex Diagnostics s.r.l., ITALY).

Student's t tests were used for the differences between (food intakes, body weights and serum total cholesterol levels) of test and control groups. Statistical significance was accepted at  $p < 0.05$ .

## RESULTS

Table 1 shows constituents of pomegranate seeds and juice, which were analyzed separately. Reducing sugar and Vitamin C were found in squeezed fluid but not in crushed seeds. However, there were flavonoids and tannin (Antioxidants) in both fluid and seeds.

Table 1. Chemical constituents of pomegranate seeds and fluid

| Chemical Constituents | Pomegranate Seeds | Pomegranate Fluid |
|-----------------------|-------------------|-------------------|
| Alkaloid              | (-)               | (-)               |
| Terpene               | (-)               | (+)               |
| Reducing sugar        | (-)               | (+)               |
| Flavonoid             | (+)               | (+)               |
| Steroid               | (-)               | (-)               |
| Glycoside             | (+)               | (+)               |
| Tannin                | (+)               | (+)               |
| Saponin               | (+)               | (-)               |
| Amino acid            | (+)               | (+)               |
| Vitamin C             | (-)               | (+)               |
| (+)                   | Present           | (-) Absent        |

Figure 1 shows three-day food intake of both groups during the period of PJ supplementation. Food intake of all rats was lower during the period of high cholesterol diet feeding than that of usual food feeding ( $21.9 \pm 5.6$ g/day vs  $25.1 \pm 3.4$ g/day,  $p > 0.05$ ), but not shown in the figure. Food intakes of test and control groups were not different during first month of PJ supplementation ( $18.3 \pm 4.1$ g/day vs  $24.1 \pm 2.6$ g/day,  $p > 0.05$ ) but during second and third month of PJ supplementation, food intake of test group were significantly lower than those of test group ( $16.6 \pm 2.6$ g/day vs  $25.3 \pm 5.6$ g/day,  $17.8 \pm 3.4$ g/day vs  $26.8 \pm 4.8$  g/day,  $p < 0.005$  for each).

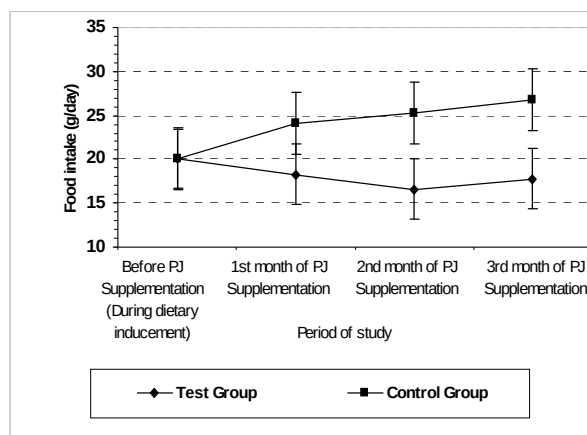


Fig. 1. Food intake (g/day) of test and control groups before and after PJ supplementation

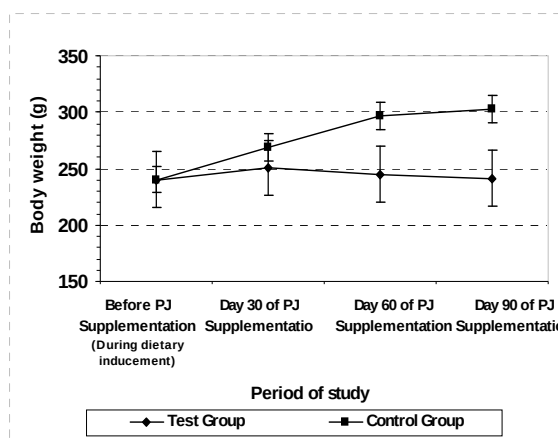


Fig.2. Body weights of test and control groups before and after PJ supplementation

Figure 2 shows body weights of test and control groups on Day 30, Day 60 and Day 90 of PJ supplementation. Body weights of test group were lower than those of control group ( $250.6 \pm 34.3$  vs  $268.8 \pm 11.8$  g) although it was not statistically different ( $p > 0.05$ ) but significantly lower body weights of test group were found in Day 60 and Day 90 of PJ supplementation ( $245.1 \pm 22.9$  vs  $296.3 \pm 11.4$  g) and ( $241.3 \pm 37.3$  vs  $302.8 \pm 12.6$  g),  $p < 0.05$  for each).

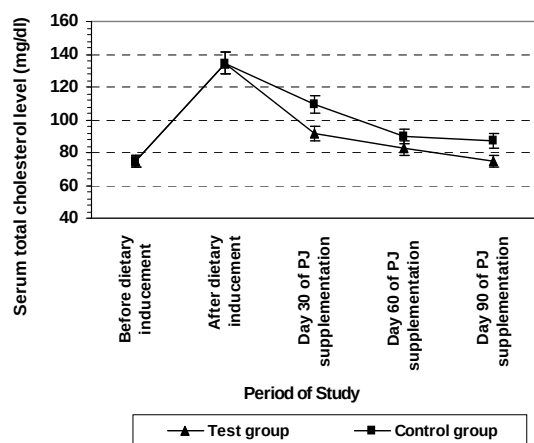


Fig. 3. Serum total cholesterol levels of test and control groups before and after PJ supplementation

Figure 3 shows serum total cholesterol levels of both groups. Serum total cholesterol levels of the test group were lower than those of the control group on Day 30 ( $91.44 \pm 22.9$  mg/dl vs  $109.4 \pm 27.19$  mg/dl), and on Day 60 ( $82.6 \pm 17.3$  mg/dl vs  $89.7 \pm 13.96$  mg / dl) of PJ supplementation,

although they were not statistically significant ( $p > 0.05$  for both). On Day 90 of supplementation, serum total cholesterol levels of test group was significantly lower than those of control group ( $74.4 \pm 10.11$  mg/dl vs  $86.8 \pm 10.52$  mg/dl), ( $p = 0.043$ ).

## DISCUSSION

The pomegranates have been shown to contain powerful antioxidant compounds as well as macro and micronutrients. Edible parts of pomegranate fruit (about 50% of total fruit weight) comprise 80% juice and 20% seeds. Pomegranate juice (PJ) was shown recently its impressive antioxidative properties. Fresh juice contains 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid, and polyphenolic flavonoids (tannins, anthocyanins, ellagic acid derivatives). Pomegranate seeds are rich in sources of crude fibers, pectin, and sugars. Pressing the whole fruit results in much higher contents of pericarp polyphenols in juice. The seed oil consists of 63.5% punicic acid—a rare trans 18-carbon fatty acid (structurally related to conjugated linolenic acid) [13, 14].

According to USDA National Nutrient Database, nutritive values of 100g edible portion of pomegranate contain 15% carbohydrate, 1.6% protein, 0.7% minerals, 5% fibers, 10 mg calcium, 0.3 mg iron, 70mg phosphorus and 16mg vitamin C. The principal amino acids are glutamic and aspartic acids.

In the study, in comparison of food intake of the controls, that the test group's food intake was significantly lower in second and third month of PJ supplementation might be due to less energy getting from PJ than from food despite the presence of macronutrients in PJ. This finding was not found in a study where long-term oral administration of pomegranate flower extract could not reduce body weight in Zucker diabetes fatty rats and appetite suppressing activity of PJ has not been proved yet [15].

Cholesterol, the characteristic of alcohol of animal tissues, performs a number of essential functions in the body. Thus, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. Cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol as well as de novo synthesis by extrahepatic tissues as well as by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma proteins sent to the peripheral tissues. In humans, the balance between cholesterol influx and efflux is not precise, resulting in gradual deposition of cholesterol in the tissue, particularly in the endothelial linings of blood vessels.

There are two forms of cholesterol: circulating and cellular cholesterol. Serum total cholesterol, measured in this study is a kind of circulating cholesterol. HMG CoA (3-hydroxy-3-methylglutaryl CoA) reductase is the major rate-limiting enzyme for cholesterol biosynthesis and its gene expression is controlled by a transcription factor (Sterol regulatory element binding protein or SREBP). Other two enzymes: acyl CoA cholesterol acyltransferase (ACAT) and lecithin cholesterol acyltransferase (LCAT) are to change the free cholesterol to the storage forms. The activity of ACAT is enhanced in the presence of increased intracellular cholesterol.

A group of researchers from Iran showed that after 8-week consumption of concentrated pomegranate juice, plasma total cholesterol and LDL-cholesterol levels were lower significantly ( $p < 0.006$  for each) in diabetic patients with hyperlipidemia than those of the controls but mechanism of hypolipidemia could not be explained [16]. Fuhrman found that macrophage cholesterol biosynthesis was inhibited by 50% ( $p < 0.01$ ) after cell incubation with pomegranate juice [17]. This inhibition, however, was not

mediated at the HMG CoA reductase level along the biosynthetic pathway. In a study of *in vivo* and *vitro*, long term oral administration of pomegranate flower extract (500mg/Kg) reduced cardiac triglyceride (TG) content, accompanied by a decrease in plasma levels of TG and total cholesterol in Zucker diabetic fatty rats. They suggested that improvement of cardiac lipid metabolism in these rats was due to activation of PPAR- $\alpha$  (peroxisome proliferation-activated receptors-  $\alpha$ ) and thereby lowering circulating lipid and inhibiting its cardiac uptake [15].

Hypolipidemic effect of PJ may be due to polyphenolic flavonoids which are abundantly present in it. *In vitro* studies of Chen et al and Chang et al. [18, 19], inhibitory effects of polyphenolic flavonoids and tannin derivatives on HMG CoA reductase of vero cells, a cell line obtained from kidneys of African green monkeys, were found. Mullen et al. [20] hypothesized that maturation of sterol regulatory element binding proteins (SREBPs) and SREBP-regulated genes in HepG2 cells by isoflavones produce an increase in surface LDL receptor expression that increases the clearance of plasma cholesterol, thus decreasing plasma cholesterol levels.

*In vivo* experimental study of Bok et al. [21] showed lipid lowering efficacy of flavonoids in high cholesterol fed rats. They found that flavonoids supplementation on male rats with a high cholesterol diet significantly lowered the levels of plasma cholesterol ( $2.44 \pm 0.59$  vs  $3.8 \pm 0.28$  mmol/L,  $p < 0.05$ ), hepatic cholesterol ( $0.143 \pm 0.017$  vs  $0.181 \pm 0.003$  mmol/g,  $p < 0.05$  and hepatic triglycerides ( $0.069 \pm 0.007$  vs  $0.095 \pm 0.002$  mmol/g,  $p < 0.05$ ) compared to those of the controls. They also showed that significant reduction activities of HMG CoA and ACAT.

Vitamin C also favors cholesterol excretion (bile acids formation at the 7 $\alpha$ -hydroxylation step). According to USDA National Nutrient Database, relatively high content of

polyunsaturated fatty acids (PUFA) in PJ stimulates cholesterol excretion into the intestine and oxidation of cholesterol to bile acids and up-regulation of LDL receptors leading to distribution of cholesterol from the plasma into the tissues. Therefore, in the present study, significant reduction of serum total cholesterol levels in hypercholesterolemic rats after 90-day PJ supplementation may be due to contents of antioxidants (mainly flavonoids and tannin) and vitamin C in PJ. Furthermore, low food intake during PJ supplementation needs to be considered the fact of lowering serum total cholesterol level.

In conclusion, 90-day supplementation of PJ significantly reduces serum total cholesterol levels in hypercholesterolemic rats compared to their controls. Thus, daily consumption of PJ may prevent to increase serum total cholesterol level in human. However, as this study was carried out only on a type of laboratory animal, effect on human needs to be studied.

## ACKNOWLEDGEMENT

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## The effect of maternal malnutrition on physical growth of Wistar rats

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The study was conducted to find out the effect of maternal malnutrition during the first and third trimesters of pregnancy on body weight and length of the offsprings up to adult life. Thirty Wistar strain female rats were randomly divided into 3 groups and were allowed to become pregnant. Forty percent restriction of food intake was done during the first trimester in test I group and during the third trimester in test II group while one group, the control group, was given food *ad libitum* throughout pregnancy. The offsprings of all three groups were allowed to take food *ad libitum*. Mean birth weight, mean weaning weight, mean weaning length and mean adult length of test II group were significantly lower ( $p < 0.0001$ ) than those of the control group (4.56g vs 5.46g, 19.57 g vs 23.84 g, 14.45 mm vs 16.08 mm and 39.78 mm vs 41.15 mm respectively). However, mean birth weight (5.4 g), mean weaning weight (24.78 g), mean weaning length (16.08 mm) and mean adult length (40.82 mm) of test I group were more or less similar to those of control group but mean adult weight of test I group (263.04 g) was significantly greater ( $p < 0.004$ ) than that of the control group (237.65 g). From this study, it was found that maternal malnutrition during third trimester can cause defective physical growth of off-spring.

## INTRODUCTION

It has been reported that maternal malnutrition affected the fetal growth and development [1, 2]. In rats, a 50 % overall reduction of food intake or a decrease in protein content of 5 to 6 % from early pregnancy or throughout the pregnancy caused 20 to 30 % reduction in birth weight of offspring [3]. However, Nailsmith (1969) and Rippel *et al.* (1965) showed that in rats and pigs, moderate protein deprivation had no effect on the weight of the off-spring [4,5]. Studies by Polani (1974) indicated that about 40% birth weight variation was due to genetic factors and about 60% was due to the fetal environment such as maternal health and nutrition [6]. According to Hytten (1979), the belief widely held by nutritionists, that the fetus is vulnerable to maternal dietary inadequacy in pregnancy may be mistaken [7]. The widespread changes in the maternal homeostasis of

nutrients makes it easier for the placenta to compete with maternal tissues for supplies. Essential nutrients are acquired by the fetus via active fetus-directed transport so that in extreme conditions the fetus will acquire nutrients at the expense of the mother [7]. However, Girard *et al.* (1979) pointed out that low fetal birth weight occurred following fasting during late pregnancy in rats ( 17.5 to 21.5 days ) [8]. Seller (1987) brought to light the effect of maternal nutrition on different embryonic stages in rats. He divided prenatal development into 3 distinct phases: pre-implantation, implantation and post-implantation. He stated that alteration in nutrition does not usually affect the early pre-implantation phase but markedly affect on later two phases [9]. It was also reported that food restriction during the third trimester gives rise to low birth weight pups; and the dams which were food restricted for the first two trimesters but re-fed during third trimester showed that

in every parameter, they were comparable with control [10].

Barker's discovery in 1984 that low birth weight linked to cardiovascular disease in adulthood had led to a discovery of fetal programming. Under-nutrition during the fetus's first trimester makes obesity more likely in adulthood, perhaps because the appetite control centre in brain is programmed to over-eat and fetus undernourished later in gestation may develop fewer fat cells. That makes it harder to become fat after birth [11].

Therefore, the present study was aimed to find out the effect of maternal malnutrition during first and third trimesters of pregnancy on body weight and length (growth) of the off-springs up to adult life.

## MATERIALS AND METHODS

### Study design

It was a randomized experimental trial design.

### Materials

Wistar rats from laboratory Animal Services Division, DMR(LM) were used.

### Method

Thirty pairs of Wistar rats were randomly divided into three groups. One group was used as control. Out of 10 female rats used, eight female rats became pregnant and completed the study. They were fed diet *ad libitum*.

In another group (Test I group), out of 10 female rats, 7 became pregnant and completed the study. These pregnant rats were restricted in food intake (40% restriction) during the first week of pregnancy but later re-fed *ad libitum*.

In the other group (Test II group), out of 10 female rats, 5 became pregnant and completed the study. These pregnant rats

were given food *ad libitum* and restricted in food intake (40% restriction) during last week of pregnancy. Litter size, birth weight, weaning weight, weaning length (snout to rump length) and weekly changes in weight and length were recorded up to adult life.

To all groups, clean cages and autoclaved bedding were provided twice a week and autoclaved DMR rat diet and tap water were provided daily. The room temperature was kept at  $19 \pm 2^\circ \text{C}$ .

### Data analysis

Means of all parameters were compared by the student 't' test and the differences were considered as significant at  $p < 0.05$ .

## RESULTS

Figure 1 shows comparison of mean maternal body weight changes during pregnancy among Test I, Test II and Control groups. Weight reduction was found to coincide with food restriction period.

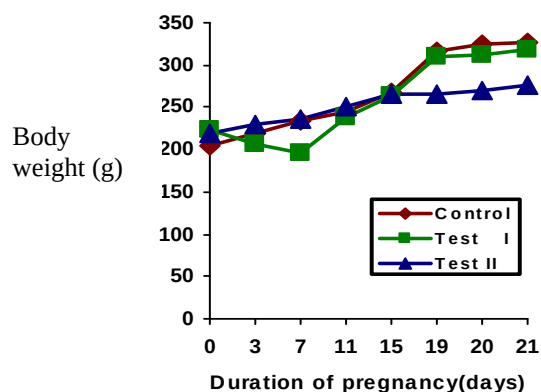


Figure 1. Maternal body weight changes during pregnancy

Mean birth weight, mean weaning weight, mean weaning length and mean adult length of off-springs of Test II group were significantly lower ( $p < 0.0001$ ) than those of control group but mean adult weight of off-springs of Test I group was significantly greater ( $p < 0.004$ ) than that of control group (Table 1).

Table 1. Comparison of mean litter size, mean birth weight, mean weaning weight, mean weaning length, mean adult weight and mean adult length of off-springs of Test I, Test II and Control groups.

| No                     | Control        | Test I           | Test II        |
|------------------------|----------------|------------------|----------------|
| 1 No. of pregnant rats | 8              | 7                | 5              |
| 2 Litter size          | 13.50 ± 2.98   | 10.86 ± 3.93     | 15.00 ± 3.16   |
| 3 No. of off-springs   | 95             | 68               | 60             |
| 4 Birth weight (gm)    | 5.40 ± 0.38    | 5.46 ± 0.59      | 4.56 ± 0.24*   |
| 5 Weaning weight (gm)  | 23.84 ± 2.99   | 24.78 ± 8.85     | 19.57 ± 4.20*  |
| 6 Weaning length (mm)  | 16.08 ± 1.17   | 16.08 ± 1.85     | 14.45 ± 1.62*  |
| 7 Adult weight (gm)    | 237.65 ± 49.86 | 263.04 ± 56.17** | 236.95 ± 43.96 |
| 8 Adult length (mm)    | 41.15 ± 2.05   | 40.82 ± 2.04     | 39.78 ± 1.62*  |

\* significant difference when compared with the control ( $p < 0.0001$ )

\*\* significant difference when compared with the control ( $p < 0.004$ )

Figure 2 shows comparison of changes in mean body weight of the off-springs of Test I and Test II groups with that of the Control groups. The off-springs of Test II group have lower weight gain.

Figure 3 shows comparison of changes in mean body length of the off-springs of Test I and Test II groups with that of the Control groups. The off-springs of Test II group have also lower mean body length changes than those of other groups.

## DISCUSSION

Regarding maternal body weight changes during pregnancy, Wistar rats given DMR stock diet *ad libitum* showed a slight increase in body weight during the first two weeks (0 to 14 days) and a marked increase in body weight during the third week (15 to 21 days). This was in accord with

Beaton's report (1954) [12] as well as Hla Hla Aye's findings [10]. Those pregnant rats during 40% overall reduction in food intake were found to be associated with marked reduction in body weight.

In our experiment, mean litter sizes of Control group, Test I group and Test II group were not significantly different. Mean birth weight of pups born from Test II group [The pregnant dams who were given restricted food intake during the last week (15 – 21 days)] was significantly lower ( $p < 0.0001$ ) than that of the control group. However mean birth weight of pups born from Test I group [The pregnant dams who were given restricted food intake during the first week (1 – 7 days)] was comparable to that of the control group. This finding was also in accord with those of Seller's report [9] and Hla Hla Aye's findings [10]. These findings may be explained by the facts suggested by Seller (1987) [9]. He stated that in the pre-implantation period the embryo acquired nutrients by diffusion and maternal nutrition does not affect the embryo development. In post-implantation period, nutrients exchange occurred across the placenta membrane and alteration of maternal nutrition could have marked effects on the fetus.

Most of the studies on the effect of maternal nutrition on the off-springs put emphasis only on the birth weight and their exploration did not extend to adult life. In our study, the effects of maternal nutrition on the off-springs were observed up to adult life and also extended to the next generation. In the study of growth, changes in both body weight and snout to rump length were main parameters to be considered. In this study, changes in both body weight and snout to rump length up to adult life of the pups of group II were lower than those of the control group. This indicates the fact that maternal malnutrition during third trimester can affect not only the birth weight but also growth of pups in later life. Although adult weight of

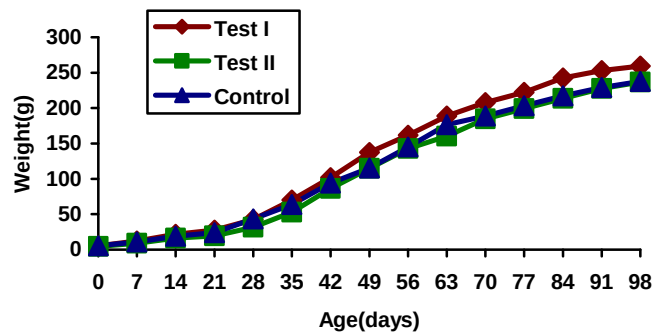


Figure 2. Comparison of changes in mean body weight of the off-springs of Test I and Test II groups with that of the Control groups

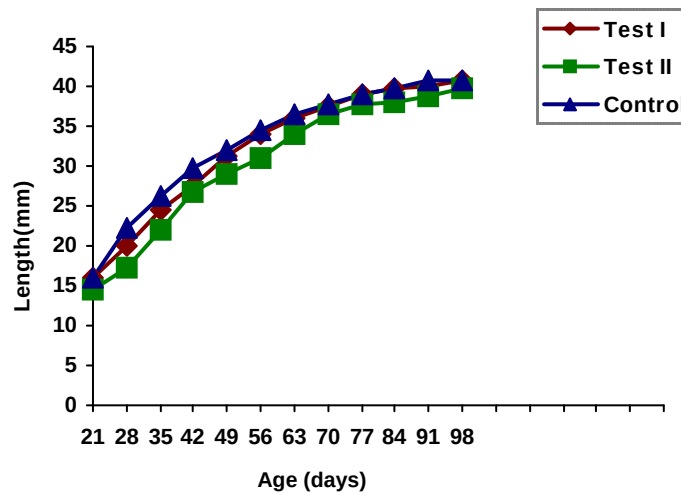


Figure 3. Comparison of changes in mean body length of the off-springs of Test I and Test II groups with that of the control groups

pups is not significantly lower than that of the control group, adult length was still significantly lower than that of control group. However, changes in both body weight and snout to rump length up to adult life of the pups of group I were more or less comparable to those of the control group although adult weight of the pups of group I was significantly greater ( $p < 0.004$ ) than that of the control group. These findings were in accord with the concept of "fetal programming" suggesting that as pregnancy progress, each month in the womb shapes health for life. As mentioned above, under-nutrition during the fetus's first trimester makes obesity more likely in adulthood, perhaps because the appetite control centre in brain is programmed to over-eat and

fetus undernourished later in gestation may develop fewer fat cells [11]. That makes it harder to become fat after birth. From this study, it was found that maternal malnutrition during third trimester could cause defective physical growth of off-spring.

#### ACKNOWLEDGEMENT

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**A preliminary study of the immune response to  
locally produced recombinant hepatitis B vaccine in adult volunteers**

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The immune response to locally produced recombinant hepatitis B vaccine was studied on 17 male adult volunteers. All subjects were negative for the hepatitis B markers namely hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs). Three doses of recombinant hepatitis B vaccine were given at 0, 1, 2 month intervals by intramuscular (IM) route. Eleven subjects were given 3 vaccinations of 20µg per dose and 6 subjects were vaccinated with 40µg of recombinant hepatitis B vaccine as the initial dose and 20µg for the subsequent second and third doses. Blood samples were collected from each subject at 1, 2, 3 month intervals and tested for the presence of anti-HBs using the SD Anti-HBs ELISA 3.0, Standard Diagnostic, Korea. A known anti-HBs positive serum (400 m IU/ml) was used as the Working Standard. Anti-HBs was present in 23.5% of subjects one month after the first dose, 52.9% after the second dose and 88.2% after the third dose. The anti-HBs values ranged from 2 - 72 m IU/ml and protective anti-HBs levels (more than 10 m IU/ml) were achieved in 76.5% of the vaccinees at the end of 3 months. From this preliminary study, it could be concluded that the locally produced recombinant hepatitis B vaccine is immunogenic in human subjects.

## INTRODUCTION

There are estimated 80 million HBV carriers in the South-East Asia Region, which are 7% of the regional population [1]. The World Health Organization have classified countries as high, intermediate and low endemic prevalence, depending on the carrier rate of the hepatitis B virus (HBV). Carrier rates of 8-20% are classified as high prevalence, 2-7% as intermediate and below 2% as low. Introduction of hepatitis B vaccine in the childhood immunization schedule is recommended for countries with the HBV carrier rate of more than 2%. In the South-East Asia region, only Nepal and Sri Lanka have prevalence rates below 2% [1]. Hepatitis B vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure [2].

Hepatitis B virus infection is hyper-endemic in Myanmar and is a major health problem in the country. A large-scale field study carried out in both lower and upper Myanmar had shown that 10.4% of the study population was seropositive for hepatitis B surface antigen (HBsAg) [3]. Subsequent studies carried out among different population groups had revealed HBsAg carrier rates of 10-12% [4].

A pilot scale production of plasma-derived hepatitis B was carried out at the Department of Medical Research (DMR) from 1990 to 1996, funded by the United Nations Development Programme and executed by the World Health Organization. Since 1997, a small-scale production of plasma-derived hepatitis B was carried out at the DMR with an annual production of approximately 100,000 pediatric doses. However, as the plasma-derived HB vaccine

was associated with the unwarranted fears by the public of acquiring blood-borne infections, production of an alternative hepatitis B vaccine was considered by the health authorities of the country.

In 2003, a recombinant hepatitis B vaccine produced from HBsAg expressed in the methylotrophic yeast *Hansenula polymorpha* was first produced at the Hepatitis B Vaccine Plant, Department of Medical Research (Lower Myanmar) with the technology transfer from the CJ Corporation, under the loan from the Economic Development and Cooperation Fund of the Republic of Korea.

Findings from the Phase 1 Clinical trial had established the safety of the candidate vaccine. This preliminary study was conducted to evaluate the immune response of the recombinant hepatitis B vaccine in adult human volunteers.

## MATERIAL AND METHODS

### *Subjects*

Seventeen adult volunteers from Ma Lit village in Hlegu Township who had participated in the Phase 1 clinical trial were further recruited for this study. The subjects were males and within the age range of 18-38 years. All the subjects were negative for hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs).

### *Candidate vaccine*

Indigenous Recombinant hepatitis B vaccine produced from HBsAg expressed in methylotrophic yeast *Hansenula polymorpha*.

### *Methods*

The subjects were divided into two groups: Group A and Group B.

#### *(i) Group A*

Group A comprised of 6 subjects and 40µg of recombinant hepatitis vaccine was given to each of the 6 subjects of Group A

as the initial dose, in the deltoid region by intramuscular route according to the 0, 1, 2 month immunization schedule. 20µg of recombinant hepatitis vaccine was given for the second and third doses.

#### *(ii) Group B*

Group B consisted of 11 subjects and three doses of 20µg of recombinant hepatitis vaccine were given to each of the subjects of Group B in the deltoid region by intramuscular route, according to the 0, 1, 2 month immunization schedule.

### *Measurement of anti-HBs*

Blood samples were collected from each subject at 1, 2, 3 months after the initial dose and tested for the presence of anti-HBs by an Enzyme-linked Immuno Sorbent Assay (SD Anti-HBs ELISA 3.0, Standard Diagnostic, Korea). Anti-HBs positive serum with a titer of 400 m IU/ml was used as the working standard.

### *Interpretation*

Seroconversion was defined as an anti-HBs concentration of more than 1 m IU/ml in a post-vaccination serum of an initially seronegative individual.

Sero-protection was interpreted as the presence of anti-HBs in concentrations of 10 m IU or more than 10 m IU/ml in a post-vaccination serum of an initially seronegative individual.

## RESULTS

Seroconversion rates of vaccinees from Group A were 16.6%, 50.0% and 83.3% one month after 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> dose of vaccination, while the seroconversion rates of subjects from Group B were 27.2%, 54.5% and 90.9% after each dose. The overall seroconversion rate of the vaccinees of this study was 23.5%, 52.9% and 88.2% one month after the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> dose of vaccination (Table 1).

Table 1. Seroconversion rates of vaccinees at different time intervals

| Time interval after 1st dose | Group A (n=6) | Group B (n=11) | Total (%)  |
|------------------------------|---------------|----------------|------------|
| 1 month                      | 1 (16.6%)     | 3 (27.2%)      | 4 (23.5%)  |
| 2 month                      | 3 (50.0%)     | 6 (54.5%)      | 9 (52.9%)  |
| 3 month                      | 5 (83.3%)     | 10 (90.9%)     | 15 (88.2%) |

Although there was seroconversion, none of the vaccinees in Group A achieved sero-protective levels of anti-HBs at 1 month after the first dose, however, 33.3% and 50.0% of the vaccinees developed protective anti-HBs titers one month after the 2<sup>nd</sup> and 3<sup>rd</sup> vaccinations. The seroprotection rates of subjects from Group B were 27.2%, 54.5% and 90.9% one month after the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> vaccinations. Overall, a total of 17.6%, 47.1% and 76.4% of the vaccinees achieved seroprotection at 1, 2 and 3 months (Table 2). One subject from Group A and one from Group B failed to seroconvert at one month after the 3<sup>rd</sup> dose.

Table 2. Seroprotection rates of vaccinees at different time interval

| Time interval after 1st dose | Group A (n=6) | Group B (n=11) | Total (%)  |
|------------------------------|---------------|----------------|------------|
| 1 month                      | 0 (0%)        | 3 (27.2%)      | 3 (17.6%)  |
| 2 month                      | 2 (33.3%)     | 6 (54.5%)      | 8 (47.1%)  |
| 3 month                      | 3 (50.0%)     | 10 (90.9%)     | 13 (76.4%) |

Anti-HBs levels of 1- >50 m IU/ml were achieved in 83.3% of vaccines from the Group A and 90.9% from the Group B. In the two study groups, anti-HBs levels were present in 88.2% of the vaccinees (Table 3).

The seroconversion rate of Group A (6 cases), who received 40µg of vaccine as the initial dose was (83.3%), and seroprotection was achieved in 50% at 3 months after the first dose. In Group B,

Table 3. Anti-HBs titers in different groups (3 months after first dose)

| Anti-HBs titer (m IU/ml) | Group A (n=6) | Group B (n=11) | Total (%) |
|--------------------------|---------------|----------------|-----------|
| 1-9                      | 2 (33.3)      | 0 (0)          | 2 (11.8)  |
| 10-30                    | 1 (16.7)      | 2 (18.2)       | 3 (17.6)  |
| 31-50                    | 1 (16.7)      | 2 (18.2)       | 3 (17.6)  |
| > 50                     | 1 (16.7)      | 6 (54.5)       | 7 (41.2)  |
| Total                    | 5 (83.3)      | 10 (90.9)      | 15 (88.2) |

the seroconversion and seroprotection rates were both 90.9%. The overall seroconversion rate was 88.2% out of which 76.5% achieved sero-protective levels of anti-HBs (Table 4).

Table 4. Seroconversion and seroprotection rates at 3 months after the first dose

| Percentage                    | Group A (n=6) | Group B (n=11) | Total      |
|-------------------------------|---------------|----------------|------------|
| Seroconversion (≥ 1 m IU/ml)  | 5 (83.3%)     | 10 (90.9%)     | 15 (88.2%) |
| Seroprotection (≥ 10 m IU/ml) | 3 (50.0%)     | 10 (90.9%)     | 13 (76.5%) |

## DISCUSSION

In this study, the mean age of the subjects was 25.41 (SD 6.20) years. The mean age of vaccinees allocated to group A was 26.83 (SD 6.49) years while the mean age of the 11 subjects in Group B was 24.64 (SD 6.20) years. Six subjects in Group A were given 40 µg of recombinant HB vaccine as the first dose followed by 20 µg for the second and third doses. Eleven subjects in Group B were given 3 doses of 20 µg of recombinant HB vaccine. The vaccines were given according to the 0, 1, 2 schedule.

Seroconversion rates (anti-HBs titer > 1m IU/ml) of vaccinees from Group A were 16.6%, 50.0% and 83.3% one month after 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> doses, while the seroconversion rates of subjects from Group B were 27.2%, 54.5% and 90.9% at similar time intervals. One subject from Group A and one from Group B failed to seroconvert at one month after the third vaccination. The overall seroconversion rates in this

study were 23.5%, 52.9% and 88.2% one month after 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> vaccinations.

Although the seroconversion rates of Group B were slightly higher than those of Group A, there was no significant difference in the seroconversion rates between the groups, indicating that there was no dose-dependency in relation to the initial dose, although dose-dependency of yeast-derived HB vaccine was reported by Just and co-authors who found the geometric mean titer of anti-HBs levels in groups receiving 20 µg doses of yeast-derived vaccine were higher than in those administered the 10 µg doses [5].

The percentage of subjects responding to the vaccines with anti-HBs values above 10 m IU/ml are of greater importance than the overall seroconversion rates since it indicates protection against the hepatitis B virus infection. In this study, protective anti-HBs titers developed in 17.6%, 47.1% and 76.4% of the vaccinees, one month after the completion of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> doses of vaccination. Various authors had reported different seroprotection rates in studies using the 0, 1, 2, immunization schedule.

Seroprotection rates of 15.4%, 66.7% and 96.8% had been reported by Wiedermann *et al.* in a study population immunized with 3 doses of 20µg doses of yeast-derived hepatitis B vaccine [6]. In a study by Scheiermann *et al.*, the seroprotection rates were 20%, 70% and 100% in 220 medical students immunized with three 20µg doses of yeast-derived vaccine [7]. Isahak and co-authors reported the seroprotection rates of 7%, 79% and 100% in Malaysian medical students vaccinated with 3 doses of Engerix B (20µg) according to the 0, 1, 6 schedule [8].

Although the seroprotection rate of the candidate recombinant hepatitis B vaccine at one month after the first dose was comparable to the findings of other studies, the seroprotection rates following the second and third doses were found to be

lower. Hepatitis B vaccines are highly immunogenic but have decreased immunogenicity associated with increasing age, obesity, smoking, presence of a chronic disease and male gender [9]. The relationship between diminished vaccine response and age, as well as other factors such as smoking and increased body mass had been observed by different authors [10, 11]. In the present study, the subjects were all males and more than 50% of them were smokers who might have contributed to the lower immunogenicity rates.

The time interval between the completion of the third dose and determination of anti-HBs was relatively short. Findings from many studies have shown that many seeming non-responders to hepatitis B vaccines must be considered as slow responders since the majority of apparent non responders develop antibodies at a later date [12]. If the anti-HBs testing was done at a later date, the two vaccinees who had failed to produce detectable levels of anti-HBs, might have seroconversion.

In conclusion, although the seroprotection rate was lower than that of other studies, the indigenous recombinant hepatitis B vaccine expressed in yeast cells of *Hansenula polymorpha* was found to be immunogenic and able to induce protective anti-HBs levels in the majority of the adult male volunteers.

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## Bacteriological aspects of some food available in Yangon area

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Bacterial pathogen contamination of 81 food samples from Pazundaung, Mingalar Taungnyunt and Pabedan Townships was carried out from June 2002 to May 2004. These samples were 26, 21, 19 and 15 of noodles, bread, biscuits and cookies respectively. The total bacterial count, total coliform and faecal coliform count were tested in all those food samples using conventional methods. Plasmid isolation was done on *Escherichia coli* isolates. In this study, the Colony Forming Units (CFU) of bacteria counted were  $>7.5 \times 10^5$  organisms/gram in 27 samples (33.33%);  $1.5 \times 10^4$  to  $7.5 \times 10^5$  CFU/gram in 26 samples (32.09 %); and  $<2$  CFU/gram in 28 samples (34.56%). Coliforms (100%) and faecal coliforms (61.53%) were identified from noodles. From bread, coliforms (71.42 %) and faecal coliforms (28.57%) were identified. From biscuits, coliforms (71.42 %) and faecal coliforms (21.05 %) were identified. Also, coliforms (46.66 %) and faecal coliforms (20.0%) were identified in cookies. From all foods tested, coliforms were isolated in 72.83% and faecal coliforms in 35.80%. *Staphylococcus* species was isolated from 46.15 % of noodles and 37.50 % of bread, however, none from biscuits and cookies. *Bacillus* species was isolated from 92%, 76.19 %, 21.05%, and 33.33% of noodles, bread, biscuits and cookies respectively. *Escherichia coli* was isolated from 100%, 71.42%, 21.05% and 53.33% of noodles, bread, biscuits and cookies respectively. Nine isolates of *E. coli* were serogrouped as O55K59 (5 isolates) and one each of O8K25, O26K50, O114K90 and O142K+. They were isolated from 6, 2 and 1 samples of noodles, bread and cookies respectively. However, *Salmonella*, *Shigella* and *Vibrios* (O1 and O139) were not isolated in this study. The plasmid size of *E. coli* isolates in this study was 23 kilo base pairs.

## INTRODUCTION

Suspect foods are those that are implicated by an attack-rate table or other epidemiological data or that have a history of being mishandled or mistreated. Potentially hazardous foods are those that readily support rapid and progressive growth of pathogens because of their properties (pH, nutrients, water activity) or that have a history of being vehicles in outbreaks of food-borne disease. The problems of food hygiene and sanitation have been reported in the various studies and it was recognized that there existed a high potential for serious health problems related to the preparation and handling of street foods. Studies from some countries

(Indonesia, Nigeria, Pune, Bombay, Columbia) confirmed both microbial and chemical contamination of food being sold by street vendors. There have been no recent epidemiological studies to suggest that street foods contribute to a significant number of food poisoning. In spite of this, there have been several documented outbreaks attributed to the consumption of Loh Shee Yum (rice noodles) bought from different hawkers. In 1981, a cholera epidemic in Pune City, India was attributed to contaminated sugar cane juice with ice. In this case, ice was found to be contaminated with *Vibrio cholerae*. In Singapore, there were 25 notified cases of food poisoning related to food center during 1987. The smallest infective dose of *Vibrio cholerae* is

$10^2$  to  $10^3$  organisms. Many reports were recorded with regard to microbiological profile of food served by street vendors in other countries [1-5].

In our country 277,871, 112,352, 4,128 and 4,477 cases of diarrhea, dysentery, typhoid and food poisoning respectively were reported in 1996. In 1999, it was reduced to 24118, 11681, and 3562 cases of diarrhoea, dysentery and food poisoning cases respectively. In Yangon Division, 1517, 26 and 7 cases of diarrhea, dysentery and food poisoning respectively, were reported in 1999 [6]. Thus, this study was carried out to know the level of contamination of food sold around Yangon area.

#### *General objective*

To identify the contamination of bacterial pathogens from wheat product foods

#### *Specific objectives*

1. To identify the bacterial count of those food
2. To define the coliforms and faecal coliforms from food
3. To diagnose the specific bacterial pathogens of food.

#### *Study period*

June 2002 to May 2004

#### *Preparation and processing of food samples for isolation of pathogens*

They were weighed as required for identification of pathogens aseptically soaked in their respective buffers, media and processed as according to the standard procedures [7].

#### *Total bacterial count*

The plate count expresses the number of all colony-forming bacteria. It is of limited value by itself, but as a supplementary test, it provides information about the amount and type of organic matter in food which may be useful in indicating the efficiency of the process of food. Specific bacterial count

was done on their selective media. The plates were incubated at 37° C and 25° C for bacteria and fungi respectively [8].

#### *Presumptive test for coliforms and faecal coliforms*

The presence of total coliforms was isolated using multiple tube method of MacConkey broth purple (double strength), incubated in waterbath at 37° C. For faecal coliforms, the usual method as described above was performed while the incubation temperature was 44.5° C [7].

#### *Isolation of Staphylococcus species, Escherichia coli, Salmonella species and Shigella species and Vibrio species*

Twenty grams of food samples were weighed aseptically and mixed well with 180 ml of phosphate buffer. For primary culture, Mannitol salt agar, MacConkey agar, Salmonella-Shigella agar and Thiosulphate Citrate-Bile sucrose (TCBS) agar plates were used and Selenite F enrichment and alkaline peptone water were used for secondary culture. The numbers of colonies identified for specific organisms multiply by the dilution factors express the value as number of cells per gram of food [8].

#### *Isolation of Bacillus species from food*

Ten grams of homogenized food were mixed thoroughly with 90 ml Mannitol egg yolk polymixin B broth. Approximately 0.02 ml of culture suspension was streaked onto Mannitol egg yolk polymixin B agar and incubated at 25-30° C for 24-48 hours. Growth of eosin pink lecithinase positive colonies after biochemical tests and Gram staining revealed *Bacillus* species..

#### *Isolation of fungi*

Twenty grams of food sample after blending aseptically were soaked and mixed with 180 ml of phosphate buffer. After 10 fold dilutions for 5-tubes, 0.02 ml each of the suspension was placed onto Sabouraud Dextrose agar. The suspension was allowed

to dry at room temperature and kept up to five days. Growth of moulds and yeasts was examined every day. The colonies were stained and examined under microscope and germ tube test was performed.

### Plasmid analysis

Rapid extraction of plasmid DNA was carried out from all enteropathogenic *Escherichia coli* isolates by the modified Birnboim procedure [9].

## RESULTS

### Total Plate count

The total bacteria count ranges from <2 to  $10^6$  (uncountable) per gram of food (Table 1).

Table 1. Total bacterial count in different kinds of food

| Bacterial count<br>CFU/gram               | Noodles<br>n=26 | Bread<br>n=21 | Biscuits<br>n=19 | Cookies<br>n=15 | Total         |
|-------------------------------------------|-----------------|---------------|------------------|-----------------|---------------|
| <2                                        | 1<br>(3.84)     | 3<br>(14.28)  | 14<br>(73.68)    | 10<br>(66.66)   | 28<br>(34.56) |
| $1.5 \times 10^4$ to<br>$7.5 \times 10^5$ | 8<br>(30.76)    | 8<br>(38.09)  | 5<br>(26.31)     | 5<br>(33.33)    | 26<br>(32.09) |
| $7.5 \times 10^5$ to<br>uncountable       | 17<br>(65.38)   | 10<br>(47.61) | 0                | 0               | 27<br>(33.33) |

Figures in parenthesis denote percentages

### Determination of presumptive coliforms and faecal coliform counts

Out of 81 food samples, coliforms and faecal coliforms were identified from 72.83 % and 35.80% respectively . Coliforms were identified from noodles (100%), bread (71.42%) biscuit (57.89%) and cookies (46.66%). Faecal coliforms were isolated from noodles (61.53%) bread (28.57%), biscuits (21.05%) and cookies (20%) (Figure1).

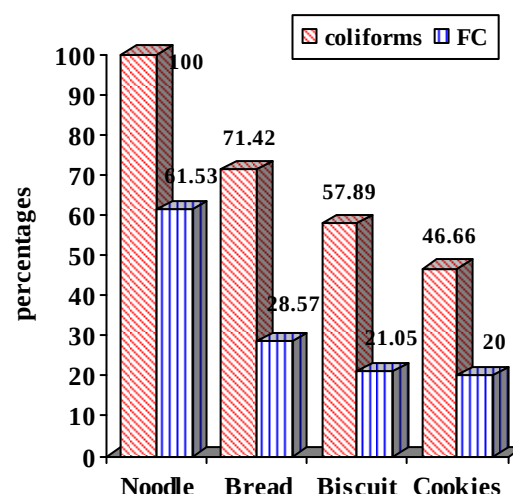


Fig 1. Coliforms and faecal coliforms from noodles, bread, biscuits and cookies from different areas of Yangon

### Distribution of *Staphylococcus* species, *Escherichia coli*, *Salmonella* species, *Shigella* species and *Vibrio* species

*Staphylococcus* species was isolated from noodles (46.15 %) and from bread (37.5%). *Bacillus* species was isolated from noodles (92%), bread (76.198%), biscuits (21.05%) and cookies (33.33%). Similarly, *E. coli* was isolated from noodles (100 %), bread (71.42%), biscuits (21.05 %) and cookies (53.33%). Serogroups of *E. coli* isolated from noodles were O55K59 (from 3 isolates) and O8K25, O26K50 and O114K90 (one isolate each). From bread samples isolates, *E. coli* serogroups were O55K59 and O142K+. *Escherichia coli* O55K59 was identified from cookies. None of the *E. coli* from biscuits could be serotyped (Table 2). *Salmonella* spp, *Shigella* spp, *Vibrio cholerae* O1 or O139 were not isolated from all the food samples.

## DISCUSSION

Recent studies indicated that cholera outbreak occurred in Mpumalanga Province, Mozambique, South Africa and in Liberia in 2003. They have shown that cholera outbreak occurred especially during the rainy season due to population movement

and the lack of safe water. Cholera and vibrio - associated diarrhoea responsible for diarrhoeal diseases are *V. cholerae* O-Group 1, non-O Group I, *V. cholerae* (non-epidemic strains), *V. parahaemolyticus*, *V. alginolyticus*, and ‘Group F vibrios’. It had been shown that cholera is food-borne transmission via street vendors, and claimed that transmission was carried through shell fish and water [3].

Table 2. Distribution of *Staphylococcus* species, *Bacillus* species, *Escherichia coli* serogroups in noodles, bread, biscuits and cookies

| Types of food    | <i>Staphylococcus</i> species | <i>Bacillus</i> species | <i>Escherichia coli</i> | serogroups                                      |
|------------------|-------------------------------|-------------------------|-------------------------|-------------------------------------------------|
| Noodles<br>n=26  | 12<br>(46.15)                 | 24<br>(92.00)           | 26<br>(100.0)           | O8K25<br>O26K50<br>O55K59<br>(3 Nos)<br>O114K90 |
| Bread<br>n=21    | 6<br>(37.50)                  | 16<br>(76.19)           | 15<br>(71.42)           | O55K59<br>O142K+                                |
| Biscuits<br>n=19 | 0                             | 4<br>(21.05)            | 4<br>(21.05)            | nil                                             |
| Cookies<br>n=15  | 0                             | 5<br>(33.33)            | 8<br>(53.33)            | O55K59                                          |
| Total<br>n=81    | 18<br>(22.22)                 | 49<br>(60.49)           | 53<br>(65.43)           | 9<br>(11.11)                                    |

Figures in parenthesis denote percentages

In this study, *Vibrio* species was isolated, however, they were not serotyped with O1 and O139 antisera. Thus, it is indicated they were termed as non-O1, non O139 strains and it may be of other species. The food to be consumed should not contain any kind of vibrios. Thus proper handling, use of clean water and utensils should be encouraged. Stool cases of children attending Yangan Children’s hospital during 2003 with diarrhoea were also isolated with *Vibrio cholerae* and it is assumed that food is one of the risk factors in transmission of this strain [10].

The epidemiology of *E. coli* that causes diarrhoea suggest that most infections are acquired through food and water. The high inoculum of *Escherichia coli* ( $10^8$ ) is

required to induce diarrhoea in healthy volunteers to support the observations. Animals (cattles and pigs) are frequently infected with *Escherichia coli* that causes diarrhoea in man. Animals and foods of animal origin have been incriminated as enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) infection [11].

Although acute diarrhoeal disease occurs at all ages, incidence is greatest among children less than five years of age and fatality is high, strikingly so in less developed countries. Besides, the so-called classic dyspepsia coli such as O26, O55, O86, O111 O127 and, many other types have been found. In this study, the serogroups O8, O26, O55, O114, O142 were identified.

In this study, *E. coli* is the most common organisms isolated with serogroup discrepancy. These serogroups encountered were within the well-known classical serogroups accepted world wide as pathogenic organisms. Thus, every food must be prepared safely not to be contaminated by pathogens especially in children’s food.

The presence of *S. aureus* indicates contamination from skin, mouth or nose of food handlers. The presence of *S. aureus* in food handlers which produced enterotoxin was shown [12]. They obtained 35 out of 102 samples (34%) had *S. aureus* and 19 out of 35 strains (54%) produced enterotoxins.

In this study, also *S. aureus* and other species were isolated representing that raw noodles are not safe for eating. Recent studies also emphasis on *B. cereus* food poisoning. In Accra, Ghana, *B. cereus* was isolated from 5.5 % of street foods [4]. In this study the isolation rate of *Bacillus* species is quite high (60.49 %). Nowadays, food safety is becoming an important public health issue. Thus, more works need to be carried out in this area [13,14].

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**Drug resistance among tuberculosis patients who sought care at private sector before taking treatment at public health service**

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As drug resistant tuberculosis (TB) is an emerging problem, delay in achieving correct diagnosis and effective treatment may lead to occurrence of drug resistant cases. This study aimed to explore the bacteriological factor of TB patients who had considerable delay while seeking treatment at private sector (general practitioners, traditional healers, self proclaimed healers) before taking treatment at public health service (TB Clinic under National TB Programme). New sputum smear positive pulmonary TB patients attending TB Centre, Yangon Division were divided into two groups as direct patients (patients attending TB Centre directly and those taking treatment at private sector for <3 weeks before reaching TB Centre) and indirect patients (patients taking treatment at private sector for >3 weeks before TB Centre). Drug susceptibility testing of *Mycobacterium tuberculosis* strains isolated from 140 direct patients and 140 indirect patients was carried out to determine bacteriological contributing factor. Resistance to at least one of anti-TB drugs was documented in 15(10.71%) direct patients and 31(22.14%) indirect patients. Mono-resistance (resistance to one drug) was noted in 6(4.29%) direct patients and 16(11.43%) indirect patients. Poly-resistance (resistance to two or more drugs) was noted in 9(6.49%) direct patients including 3(2.14%) multi-drug resistant cases (resistance to at least isoniazid and rifampicin) and 15(10.71%) indirect patients including 12(8.57%) multi-drug resistant cases. Any anti-TB drug and multi drug resistant rates were significantly higher in indirect than in direct patients ( $P<0.05$ ). This study highlighted that patients who had considerable delay while seeking treatment in private sectors before reaching public health service have greater opportunity for occurrence of drug resistance.

## INTRODUCTION

Tuberculosis (TB) is a major health problem and the world's foremost cause of death from a single infectious agent, *Mycobacterium tuberculosis*. TB causes about 8 million new cases and 2 million deaths every year. Myanmar is one of the 22 countries with highest prevalence of TB [1].

The World Health Organization (WHO) has promoted Directly Observed Therapy, Short Course (DOTS) as the policy for delivering

treatment [2]. This strategy has largely been communicated to the public health sector and implemented with various degrees of success. In Myanmar, National Tuberculosis Programme (NTP) has introduced DOTS since 1997 and all TB patients in NTP clinics are routinely treated with rifampicin, ethambutol, isoniazid, pyrazinamide and streptomycin. The DOTS detection rate of new sputum smear positive TB cases and DOTS treatment success rate were 73% and 81% respectively in 2002 [1].

Information on health expenditure suggests that poor countries have a large and growing private medical sector. Private practitioners are widely used to treat people with diseases such as TB, malaria, sexually transmitted infections, diarrhoeal diseases and acute respiratory infection [3].

The previous studies suggested that many low-income TB endemic countries with large private health sectors play a significant role in the initial stages of health seeking by symptomatic TB individuals. The large share of first contact has important implication for TB control if private practitioners follow poor diagnostic practices leading to delay in diagnosis. The delay in diagnosis is due to a delay in seeking care (patient's delay) or due to the inability of the provider to diagnose promptly (provider's delay) [4]. Lonnroth *et al.* [5] pointed that patients who had turned first to private sector were more likely to have a long provider delay than those who turned first to NTP [5].

Private health expenditure in Myanmar is 87.4% of total health expenditure [4]. A recent study which was carried out in a township in Yangon, the capital of Myanmar, pointed that 73.3% of patients at public TB centre first sought treatment at general practitioners' clinics [6].

Poor management practices in the private health sector such as delay in diagnosis and ineffective treatment dilute the epidemiological impact of DOTS programme. Such practices could also contribute to a growing incidence of drug resistant tuberculosis, which is a serious threat to TB control programme. The consequences include high morbidity and mortality, a heavy socio-economic burden and serious rate of multi-drug resistance. Thus, this study was conducted to explore the bacteriological impact in terms of occurrence of drug resistance on patients who had considerable delay while seeking treatment at private sector before taking care at public health service.

## MATERIALS AND METHODS

A cross - sectional comparative study was carried out in TB Centre, Yangon Division from October 2003 to July 2005. New sputum smear positive pulmonary TB patients irrespective of age and sex were included in the study. New smear positive cases were divided into two groups: direct and indirect patients.

### *Direct patients included*

Patients attending TB Centre without seeking treatment at private sectors and those taking treatment at private sectors for less than three weeks before reaching to the TB Centre.

### *Indirect patients included*

Patients taking treatment at private sectors for more than three weeks before reaching to the TB Centre.

*Private sector* in this study means for general practitioners, traditional medical practitioners and self proclaimed healers.

*Public health service* means for government health care service. In this study, the study area is TB Centre, Yangon Division which is a main centre of NTP.

This study was approved by the Ethical Committee of Department of Medical Research (Lower Myanmar).

### *Field Survey Procedure*

Patients who gave informed consent were included in the study and divided into direct and indirect groups according to case definition. They were interviewed using a pre-tested questionnaires designed to elicit the socio-demographic data and other contributory factors of drug resistance by trained interviewers at TB Centre. The data included age, sex, occupation, education, income, history of TB contact, how they reached TB Centre, history of taking treatment at private clinic, history of self medication, length of time between the appearance of symptoms and receiving anti-TB treatment and history of investigations done at private sector.

### Laboratory Procedure

The sputum specimens of the patients were collected and transported to Bacteriology Research Division, Department of Medical Research (LM) for isolation of *M. tuberculosis*.

The sputum specimens were decontaminated with 4% sodium hydroxide and inoculated onto egg-based Lowenstein-Jensen (LJ) medium and incubated at 37°C for 6-8 weeks. *M. tuberculosis* isolates were identified by growth rate and pigmentation.

Drug susceptibility testing of culture isolates were carried out at Reference TB Laboratory, NTP by proportion method as described in Bacteriological Methods in Laboratory Diagnosis of Tuberculosis by Tuberculosis Research Centre (ICMR) Chetput, Chennai [7].

The tested anti-TB drugs and their minimum inhibitory concentration were isoniazid (0.2 mg/L, rifapicin 40 mg/L, dihydrostreptomycin 4 mg/L and ethambutol 2 mg/L). The results were interpreted at 42 days of incubation at 37°C and the proportion of bacilli resistant to a given drug is determined by expressing the resistant portion as a percentage of total proportion tested. The result of 1% or more proportion to a above drug concentration is interpreted as resistant.

### Analysis of Results

The data were analyzed by using SPSS software. Descriptive statistics were used to describe socio-demographic data. The drug susceptibility pattern was compared between the direct and indirect groups.

## RESULTS

### 1. Demographic data of direct patients and indirect patients

The demographic data of 152 direct and 142 indirect patients is shown in Table 1. The mean age of direct patients was 33.90 (range= 14-70 years) and that of indirect

patients was 36.34 (range= 13-70 years). There were 117 men and 35 women in direct patients and 103 men and 39 women in indirect patients. Among direct patients, 62 (40.8%) were skilled workers and 68 (44.7%) had middle school education level. Among indirect patients, 76 (53.5%) were skilled workers and 55 (38.7%) had middle school education level.

Table 1. Demographic data of direct and indirect patients

| Demographic data          | Direct patients (n=152) |       | Indirect patients (n=142) |       |
|---------------------------|-------------------------|-------|---------------------------|-------|
|                           | No.                     | %     | No.                       | %     |
| <b>Age</b>                |                         |       |                           |       |
| mean age in years (range) | 33.90 ± 12.42           |       | 36.64 ± 12.31             |       |
|                           | (14-70 yrs)             |       | (13-70 yrs)               |       |
| <b>Sex</b>                |                         |       |                           |       |
| Male                      | 117                     | 77    | 103                       | 72.50 |
| Female                    | 35                      | 23    | 39                        | 27.50 |
| <b>Occupation</b>         |                         |       |                           |       |
| Unskilled worker          | 16                      | 10.50 | 24                        | 16.90 |
| Semi skilled worker       | 23                      | 15.10 | 10                        | 7.00  |
| Skilled worker            | 62                      | 40.80 | 76                        | 53.50 |
| Dependent                 | 21                      | 13.80 | 15                        | 10.60 |
| Students                  | 26                      | 17.10 | 16                        | 11.30 |
| Others                    | 4                       | 2.60  | 1                         | 0.70  |
| <b>Education</b>          |                         |       |                           |       |
| Illiterate                | 4                       | 2.60  | 5                         | 3.50  |
| Read and write            | 12                      | 7.90  | 7                         | 4.90  |
| Primary school            | 27                      | 17.80 | 23                        | 16.20 |
| Middle school             | 68                      | 44.70 | 55                        | 38.70 |
| High school               | 31                      | 20.40 | 32                        | 22.50 |
| Under graduate            | 8                       | 5.30  | 9                         | 6.30  |
| Graduate                  | 2                       | 1.30  | 11                        | 7.70  |

### 2. Drug susceptibility pattern

A total of 280 *M. tuberculosis* strains isolated from 140 direct patients and 140 indirect patients were tested for susceptibility to first line anti-TB drugs isoniazid (I), rifampicin (R), streptomycin (S) and ethambutol (E). One hundred and twenty five *M. tuberculosis* strains isolated from direct patients and 109 *M. tuberculosis* strains isolated from indirect patients were sensitive to all tested anti-TB drugs. Resistance to at least one of anti-TB drugs was documented in 15 (10.71%) of direct

patients and 31(22.14%) of indirect patients. Mono-resistance (resistance to one drug) was noted in 6(4.29%) of direct patients and 16(11.43%) of indirect patients. Poly-resistance (resistance to 2 or more drugs) was noted in 9(6.43%) of direct patients and 15(10.71%) of indirect patients.

Among the drug resistant strains isolated from direct patients, 3(2.14%) isolates were resistant to I and R, 5(3.57%) were resistant to S and I, 1(0.71%) isolate was resistant to S and R and 6(4.29%) were resistant to I. Of the mono-resistant strains of indirect patients, 13(9.29%) isolates were resistant to I, 2(1.43%) were resistant to R and 1(0.71%) isolate was resistant to E. Of poly-resistant strains of indirect patients 10(7.14%) isolates were resistant to I and R, 2(1.43%) isolates were resistant to S, I and R, 2(1.43%) isolates were resistant to S and R and 1(0.71%) isolate was resistant to S and I.

Multi-drug resistant strains were isolated (resistant to at least isoniazid and rifampicin) in 15(5.36%) of both 140 direct and 140 indirect patients comprising of 3(2.14%) direct patients and 12(8.57%) indirect patients.

Total resistance of individual anti-tuberculosis drug were: I (14, 10%), S (6, 4.29%), R (4, 2.86%) of direct patients and I (26, 18.57%), S (5, 3.57%), R (16, 11.43%), E (0.71%) of indirect patients( Table 2).

### 3. Comparison of any anti-TB drug resistance between direct and indirect patients

Any anti-TB drug resistant rate in indirect patients is significantly higher than that of direct patients.(P =0.01, chi square = 6.657) (Table 3).

### 4. Comparison of Multi- drug resistant TB between direct and indirect patients

MDR-TB rate in indirect patients is significantly higher than that of direct patients. (P = 0.02, chi square = 5.71) (Table 4).

Table 2. Drug resistant pattern of direct and indirect patients

| Anti-TB drug                     | Direct Patients<br>(n=140) | Indirect Patients<br>(n=140) |
|----------------------------------|----------------------------|------------------------------|
| I                                | 6 (4.29%)                  | 13 ( 9.29%)                  |
| R                                | -                          | 2 ( 1.43%)                   |
| E                                | -                          | 1 ( 0.71%)                   |
| S                                | -                          | -                            |
| IR                               | 3 (2.14%)                  | 10 (7.14%)                   |
| SI                               | 5 (3.57%)                  | 1 (0.71%)                    |
| SR                               | 1 (0.71%)                  | 2 (1.43%)                    |
| SIR                              | -                          | 2 (1.43%)                    |
| Any-anti TB<br>Drug<br>resistant | 15 (10.71%)                | 31 (22.14%)                  |
| MDR-TB                           | 3 ( 2.14%)                 | 12 (8.57%)                   |
| Mono-<br>resistance              | 6 (4.29%)                  | 16 (11.43%)                  |
| Poly-<br>resistance              | 9 (6.43%)                  | 15 (10.71%)                  |

I= isoniazid, R= Rifampicin, E= Ethambutol, S= Streptomycin  
MDR-TB= Multi-drug resistant TB

Table 3. Any-anti TB drug resistant rate between direct and indirect patients

| Drug Susceptibility | Direct Patients | Indirect Patients | Total |
|---------------------|-----------------|-------------------|-------|
| Resistant patients  | 15              | 31                | 46    |
| Sensitive patients  | 125             | 109               | 234   |
| Total               | 140             | 140               | 280   |

P = 0.01, chi square = 6.657

### 5. Relevant history of indirect patients

One twenty one (85.21%) of indirect patients sought treatment at General Practitioners'(GP) clinics before seeking care at NTP. The mean of duration of treatment at private sector was 40.79 days with the range of 22 days to 2 years. Six (4.2%) patients had anti-TB treatment in private clinics. The commonest investigation done at private clinics was Chest X ray 71(50%). Sputum smear examination was carried out in 30 (21.13%) patients. Twenty four (16.9%) patients had history of tuberculosis in their close contacts. Among them, 13(54.17%) took treatment from private clinics and 7(29.17%) sought treatment from NTP.

Table 4. Multi-drug resistant TB rate between direct and indirect patients

| Drug Susceptibility | Direct Patients | Indirect Patients | Total |
|---------------------|-----------------|-------------------|-------|
| MDR-TB patients     | 3               | 12                | 15    |
| Non-MDR TB patients | 137             | 128               | 205   |
| Total               | 140             | 140               | 280   |

P = 0.02, chi square = 5.71

## DISCUSSION

Spread of drug resistant TB and disastrous rate of HIV-TB co-infection pose a serious threat to TB Control Programmes around the world. The prevalence of MDR-TB and any anti-TB drug resistance among new sputum smear positive TB patients at Union Tuberculosis Institute, Yangon were 5% and 32.4% respectively in 2002 [8]. Most disease control programmes are designed to deliver exclusively through government channel. Many of the notified cases have come to NTP after seeking care from one or more private providers. Private health sectors are often more preferred by patients because of convenient hours and more personalized service. They can offer a variety of services and often more accessible to the general population and are frequently the first contact with health system when a person feels ill [9].

A recent study in Yangon, Myanmar showed that 73.3% of patients first sought treatment at two to five general practitioners' clinics before seeking care at township TB Centre and the duration of treatment was ranging from 2 days to one and half year [6]. Delay in achieving correct diagnosis and effective treatment may lead to development of drug resistance [10].

The present study showed that MDR-TB rate of all new smear positive pulmonary TB patients (140 direct and 140 indirect patients) was 5.36 % ( 15/280) and any anti-TB drug resistance rate was 16.43% ( 46/280). In spite of a decrease in any anti-

TB drug resistant rate, MDR-TB rate is almost the same when it is compared to 2002 study. These findings pointed that drug resistance still plays as a serious threat to NTP.

The most common resistant drug of direct patients was isoniazid (14, 10%) and the second resistant drug was streptomycin (6, 4.29%). Among indirect patients, the most common resistant drug was isoniazid (26, 18.57%) and the second was rifampicin (16, 11.43%). Rifampicin and isoniazid are the main bactericidal drugs and their resistance accounts for multi drug resistance (Table 2).

Demographic data of direct and indirect patients showed that the mean age of the direct patients and indirect patients were 33.0 years and 36.63 years respectively and most of the patients were within the working age group (20-59 years). Men were found to be more affected than women comprising of a large percentage of skilled workers (Table 1). Therefore, the impact of TB on that population leads to a big socio-economic burden. Moreover, there is a greater risk of transmission of TB to the community and even worse if the disease is hard to treat drug resistant case.

The relevant history of indirect patients documented that general practitioner's clinic was the most common place for first medical consultation before seeking care at NTP. The mean duration of treatment at private sector was 40.79 days ranging from 22 days to two years. Six (4.2%) of these patients have been treated with anti- TB drugs for less than one month by private practitioners. The most common investigation done at private clinics was chest X-ray (50%) and sputum examination was done only on 21.13% cases. Sputum microscopy is the main diagnostic tool for TB and categorization of patients for choice of appropriate treatment regimen is depending upon sputum smear results. Therefore, there are reasons to be delay and inadequacy in anti- TB treatment and diagnosis of private practitioners who have not kept current anti-TB therapy.

Table 3 and 4 showed that both any anti-TB drug resistance and MDR-TB rate were significantly higher in indirect patients than those of direct patients. The mean duration of treatment at private clinics was 40.79 days with the range of 22 days to 2 years. These findings indicated that indirect patients who had more delay in achieving anti-TB treatment than direct patients attending NTP directly or after being treated at private clinics for less than three weeks are more likely to be resistant to anti-TB drugs. In order to shorten the provider's delay, all medical practitioners especially in the private sectors, should be aware of the importance of early diagnosis and proper management of TB. Linking private and public sectors in TB treatment is an important task for TB Control Programmes to improve treatment outcome of TB patients who initially seek care in private sector.

The present study highlighted that drug resistance plays as a bacteriological factor for patients who have considerable delay while seeking treatment at private sector before reaching public health service. As NTP Myanmar is encouraging a private-public partnership in implementation of DOTS with the efforts like continuing medical education program, training of GPs on DOTS management, promoting of proper referral system between NTP and GP, further studies should be carried out in future to access the success of these efforts among the private practitioners..

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**Usefulness of bronze containers for decontamination of  
Gram - negative and Gram - positive bacteria from water**

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Bronze containers of different sizes with different composition of copper have beneficial effects in decontaminating different bacteria from water at different durations. Experiments revealed that Gram- negative bacteria in water were no longer detected after storage in bronze containers within 24 hours. The experiment was conducted by using bronze containers of different capacities (50 ml-500 ml). Sterilized distilled water was seeded with  $10^5$ - $10^7$  organisms per ml each of *Escherichia coli* (ATCC 25922), *Salmonella typhi*, *Proteus morganii*, *Vibrio cholerae* *Staphylococcus aureus* and *Bacillus subtilis* from overnight broth cultures. Bacteria count was done hourly by using respective selective media plates such as MacConkey agar for *E.coli* and *S .typhi* and TCBS agar for *V. cholerae* and nutrient agar for *S. aureus* and *B. subtilis* by the streak method. The plates were incubated at 37°C overnight and colony count was calculated. It was observed that all Gram-negative bacteria tested were not recovered after 4 hours of storage. The growth of bacteria depends on the type of bronze used.

## INTRODUCTION

It has been known since a long time ago that silver, copper, and zinc are commonly known elements with antibacterial and antifungal properties. Since silver coated materials have been found inappropriate in some applications, attempts have been made to investigate the antibacterial properties of copper-vermiculite [1].

Antibacterial and antifungal materials have attracted great interest in recent years because of their ability to inhibit the growth of microbes. Potential uses of antibacterial and antifungal materials include those for household, industrial and public facilities. Appropriate release of antibacterial metallic ions from composite can effectively inhibit growth of harmful microbes. Compared to the organic antibacterial material, ideal inorganic antibacterial material may have advantages such as long lasting action, chemical stability, thermal resistance,

safety, protection from drug resistance, and effectiveness against a broad spectrum of bacteria or fungi [2].

In Myanmar, bronze containers are used as household utensils in many ways and among them bronze cups are used as drinking cups, which are placed on top of earthen pots used for storage of drinking water in both rural and urban areas. Thus, this research aimed to investigate the decontamination of bacteria from water by using bronze containers.

## MATERIALS AND METHODS

### *Types of bronze containers used*

Four different kinds of Myanmar bronze containers were used.

### *Other containers*

Stainless steel containers, glass beakers, porcelain basins and chinaware were used.

### Bacteria used as contaminants

- (1) *Escherichia coli* ATCC25922
- (2) *Proteus morganii* (BIKEN)
- (3) *Salmonella typhi* (BIKEN)
- (4) *Vibrio parahaemolyticus*
- (5) *Staphylococcus aureus*
- (6) *Bacillus subtilis*

### Size of inoculum

Different amounts of water were used with respect to the size of containers. The number of bacteria in an overnight broth culture was calculated from its density by using the spectrophotometer at the wavelength of 450 nm as well as by plate dilution method. The water was allowed to contaminate with respective bacteria from overnight broth culture to obtain the concentration of  $10^6$  to  $10^7$  CFU/ml.

### Determination of the rate of decontamination

The contaminated water in different containers was kept at 25°C (RT) with an aluminium foil cover. One milliliter of water from each tested container was collected hourly up to six hours and again at 24 hours (one day) up to 5 days. Plating of samples was done on respective media and incubated at 37°C overnight. The colony forming units per ml were then calculated.

## RESULTS AND DISCUSSION

As shown in Table 1, Gram-negative bacteria count in water from bronze containers decreased hourly. In contrast, the rate of reduction of Gram-positive bacteria is slower than that of the Gram-negative bacteria. In experiments of using glass beakers, porcelain basins and stainless steel containers, the growth of bacteria after 24 hours was not significant and bacteria count was not decreased from the initial count. However, there was a 10-fold decrease in bacterial count when using earthenware (chinaware), and 20-fold

Table 1. Decontamination rate of bacteria after storage in bronze containers

| Time (hour) | Gram-negative bacteria |                 |                    |                            | Gram-positive bacteria |                    |
|-------------|------------------------|-----------------|--------------------|----------------------------|------------------------|--------------------|
|             | <i>E. coli</i>         | <i>S. typhi</i> | <i>P. morganii</i> | <i>V. parahaemolyticus</i> | <i>S. aureus</i>       | <i>B. subtilis</i> |
| 0           | $10^6$                 | $10^6$          | $10^7$             | 120                        | $10^6$                 | $10^6$             |
| 1           | 150                    | 20              | 50                 | 100                        | 1250                   | $10^6$             |
| 2           | 100                    | 18              | 40                 | 0                          | $10^3$                 | $10^6$             |
| 3           | 0                      | 9               | 20                 | 0                          | $10^3$                 | $10^6$             |
| 4           | 0                      | 1               | 0                  | 0                          | $10^3$                 | $10^6$             |
| 5           | 0                      | 0               | 0                  | 0                          | $10^3$                 | $10^6$             |
| 6           | 0                      | 0               | 0                  | 0                          | 99                     | $10^6$             |
| 24          | 0                      | 0               | 0                  | 0                          | 55                     | 0                  |

decrease when a bronze spoon was dipped in the glassware container for 4-8 hours (depending on the type of spoon). Moreover, the bacteria in water using stainless steel containers survived up to 45 days. As shown in Table 1, the bacterial count decreased hourly for Gram-negative bacteria. Thus, bronze containers are effective for the reduction of bacteria. However, Gram-positive cocci *Staphylococcus aureus* and *Bacillus subtilis* (saprophyte) took longer time than 4 hours, but the bacteria count decreased ten-fold. At the same time, when the contaminated organisms were higher than  $10^6$  organisms per ml it took more than 24 hours to be killed. Similarly, the decontamination effect of bronze depends on type of different utensils. Experiments also show that bronze could eliminate Gram-negative bacteria within 4 hours compared to Gram-positive bacteria. It will be the best container for using water storage as it could inhibit the growth of bacteria. Thus, this experiment shows that bronze containers are very useful to decontaminate pathogenic Gram-negative bacteria (water and food borne infectious agents). It has been reported by other workers [3,4] that bronze has antibacterial activity, and the benefits obtained from bronze materials may be useful tools for preventing water-borne infections in many ways for mankind. Copper is a naturally hygienic metal that slows down the growth of coliforms [5,6].

It has important potential application in food preparation, hospitals, making of coins (biocidal copper), door knobs and plumbing systems.

Bronze=Copper+ Zinc

Bronze=Copper+ Tin

Cupro nickel=Copper+Nickel

### ACKNOWLEDGEMENTS

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Ministry of Health, Yangon, Myanmar

# MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

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