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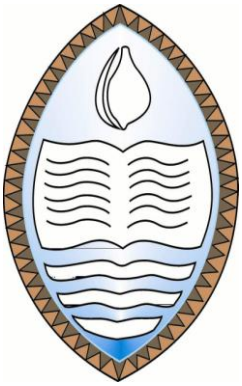

SCHOOL OF MEDICINE AND HEALTH SCIENCES
UNIVERSITY OF PAPUA NEW GUINEA

DIVISIONS OF BASIC MEDICAL SCIENCES, HEALTH SCIENCES AND DENTISTRY

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Divisions of Basic and Health Sciences, University of Papua New Guinea

November 2005

Volume 3

Contents

A STUDY OF TRACE ELEMENTS IN ARECA NUTS FROM PAPUA NEW GUINEA

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Introduction:

The *areca nut*, or the more commonly known '*betel nut*', is the seed of the betel palm or *Areca catechu*, a species of palm tree. The areca palm fruit contain the familiar, edible, brownish-red nut [1]. Areca nut contains bioactive alkaloids – the most important of which are arecaine and arecoline. Arecoline is chemically similar in structure to *Acetylcholine*, and it also has the same principle site of action as acetylcholine [1]. The alkaloids contained in betel nut have anti-muscarinic effects on smooth muscle [1], inhibit GABA receptors and have widespread effects in the body, including actions on the brain, cardiovascular system, lungs, gastrointestinal tract, and pancreas. Nitrosated derivatives of arecal alkaloids have proven to be carcinogens, and are also diabetogenic in mice [2].

It is estimated that 10-20% of the world's population (~ 600 million people) chew areca nut in some form often mixed with betel quid (pan) [3]. Betel nut chewing is a part of many cultures, especially in the Asian region and in Pacific countries like Papua New Guinea (PNG) and the Solomon Islands. The preparation technique varies from region to region, and the practice has significance in social and traditional aspects of these societies. Betel-quid or betel nut chewing results in exposure to areca nut alkaloids, N-nitroso-compounds formed during chewing, polyphenols, and trace elements. The International Agency for Research on Cancer (IARC) regards areca nut to be a known human carcinogen and there is strong evidence that chewing of areca nut causes oral submucous fibrosis, a precancerous condition in humans, and sufficient evidence of carcinogenicity in experimental animals [4].

Trace elements are elements whose quantities are small but whose contribution is enormous and essential for the body. There are seven (7) essential trace elements which have been described in humans: chromium, copper, cobalt, iodine, iron, selenium, and zinc. Trace elements play key roles, either individually or in combination, in several important functions of the body, which include maintaining the blood sugar level, thyroid hormone production, red cell production, and more importantly playing vital roles in the immune system. The immune system may not function at its fullest potential without the essential trace elements, which also have to be available to the body in adequate amounts. Trace elements are available to the body mainly through the diet and different dietary habits. One habit that may or may not expose people to trace elements is betel (areca) nut chewing, which is practiced mainly in South East Asia and the Western Pacific regions (including Papua New Guinea).

This study of areca nuts focused on the trace elements: copper, iron, manganese, selenium, zinc, and the mineral magnesium. Previous studies, which have attempted to link cancer and copper content in betel nut, have identified high concentrations of copper in betel nuts [5 & 6].

This study was done in an attempt to evaluate comprehensively the trace elements in areca nuts from Papua New Guinea. The aim was to determine some trace elements in areca nuts from different regions of Papua New Guinea. Results from the study would then allow us to speculate on the possible status of the immune system of the chewers and the implications of immunodeficient states on the pathogenesis of infection among them.

Materials and Method:

Areca nuts were randomly collected from four different regions of PNG: Central Province (CP), Gulf Province (GP), Morobe Province (MP) and North Solomons Province (NSP). About 15 to 20 unripe, green areca nuts were collected from the *areca catechu* species of areca palm. Analysis of areca nut samples was carried out at the National Analysis Laboratory (NAL-Unitech, Lae). The analysis of trace elements in areca nut was done using standard NAL methods for the analysis of trace metals [7].

The areca nut was prepared first by dehusking, grinding, homogenizing, drying (either air-dried or oven dried depending on the trace element to be tested [7]), and finally pulverizing (powdered). Two grams of the test samples were then digested overnight in a fume cupboard with a cocktail of strong acids (16N nitric acid, 60% perchlorate, and 36N sulphuric acid [7]). The acids were then evaporated, and 5-mls of the digested samples were then poured into 100-ml volumetric flasks, made up to the mark with ultra pure water and stored. The samples were then run using the Inductively Coupled Plasma (ICP) system and the Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) for copper, iron, manganese, magnesium, and zinc concentrations [7]. The results were then recorded. The samples were run using NAL standards for total metals. The analysis of selenium was done using the Atomic Absorption Spectrophotometer (AAS) Hydride System and the concentration of selenium was recorded [7]. The samples for the analysis of selenium were run using NAL standards for selenium.

Results and Discussion:

The analysis of areca nut samples showed the presence of trace elements in areca nuts, from various regions of Papua New Guinea, in varying amounts. This result agrees with the general view of previous research done to establish the constituents of areca nut [4].

The results also demonstrated that iron was more abundant in all the areca nut samples, from all the four regions, when compared to the amounts of copper, manganese, selenium, and zinc. We did not study the bioavailability of iron to chewers from areca nut. However, if the high iron in areca nut, found in this study, increased the iron content in chewers, it could adversely lead to deposition of excess iron in tissues resulting in tissue injury (MP 56μg/g, CP 50μg/g, GP 41μg/g, and NSP 35μg/g). However, this high amount could also be useful in the treatment of iron deficiencies disorders, such as iron deficiency anaemia.

The concentrations of copper and zinc in all the betel nut samples were relatively similar and in the range of 15 μg/g to 20 μg/g (For copper, GP 15μg/g, CP 15μg/g, MP 18μg/g, NSP 17μg/g; For zinc, GP 15μg/g, CP 17μg/g, MP 17μg/g, and NSP 19μg/g). The values of copper and zinc found in the betel nut presently could lead to excess of these elements in chewers. A higher value of either will lead to a lower value of the other with adverse consequences. Excess copper will lead to liver dysfunction, while a decrease in the body concentration of zinc will lead to adverse immune consequences.

The concentrations of manganese in all the samples were relatively similar except for the NSP sample, which had a higher concentration of 46 μg/g (as compared to 20 μg/g, 11 μg/g, and 8.6 μg/g for Gulf, Central, and Morobe Provinces, respectively). Altered values of manganese also play important role in compromising the immune system of the body [8].

The concentration of selenium in all the betel nut samples were all less than 1 μg/g except for the sample from Central Province, which had a selenium concentration of about 4 μg/g. Selenium plays a lot of role in the body's defence mechanism [9]. It is therefore necessary that the concentration be kept normal for an adequate function of the immune system. The present study reveals the presence of selenium in all the samples. We do not know how this affects the overall concentration of selenium in the body and therefore the concentration of the CD4 T-cells in the body (Selenium is required for activity of the CD4 T-cell enzyme glutathione peroxidase [9]).

The concentration percentages of magnesium in all the betel nut samples were in the range of 0.08 % – 0.09 % w/w per 100g of betel nut. This could be influencing the total body concentration of magnesium with adverse consequences [10].

Conclusion:

This study has revealed some interesting data about the availability of trace elements in areca nuts from Papua New Guinea. Specifically, we discovered the following:

Iron was found in all the betel nut samples from all the four regions of Papua New Guinea, and the concentration of iron was higher in betel nuts from Morobe Province, followed by Central Province. Copper and zinc was found in equal amounts in all the areca nuts. Areca nuts from North Solomon Province had significantly higher manganese values than those from the other regions. Selenium was found in all the samples of areca nuts from all the regions. Areca nuts from the Central Province had a significantly higher selenium value. Magnesium was found in identical concentrations in all the areca nuts.

This is the first study to comprehensively look into the trace elements profile of areca nuts in Papua New Guinea. The role of these elements in homeostasis has been briefly outlined[10]. Also highlighted were the consequences that might occur in situations of higher than normal concentration of these elements, particularly with regard to the immune system.

Further research needs to be conducted to establish the role that trace elements in betel nuts may play in the immune system and / or pathogenesis of infection. This will serve to broaden the scope of this work and allow for a more comprehensive conclusion.

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SCREENING PNG PLANTS FOR ANTI-TB ACTIVITY

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Background and Significance

Over one-third of the population of the world is infected with TB. Around 9 million people develop TB disease every year. Out of these, 4 million are sputum smear positive cases, and hence are infectious. More than 2 million people die due to TB every year, i.e. more than 5,000 people per day. TB kills more adults than any other infectious disease. 98% of these deaths are in the developing world. [1]

According to WHO estimates, more than 14,000 people develop TB (of all types) every year in Papua New Guinea. Over 6,000 of these patients are of the infectious type. Each infectious patient spreads TB to at least 10-15 uninfected persons every year. *Almost 8-10 people die due to TB every day in our country, and about 3000 every year, needlessly, although we have very effective drugs to treat this dreadful disease.* The national burden of TB is worsening rapidly due to poor DOTS implementation *in addition to* rise in multi-drug resistant cases. [1, 2]

Multidrug resistance is a growing clinical problem, with strains of *Mycobacterium tuberculosis* exhibiting resistance to 11 or more antimicrobial agents has been described. [3] Resistance has been observed in all the first line TB drugs such as rifampicin, ethambutol, pyrazinamide and isoniazid[4] These posses a treat to the human race as TB is seen on the rise again with approximately 3 million deaths annually. The lifetime risks of active TB are 5% to 10% among those without HIV infection and 50% in those with HIV infection.

The developing world account for about 95% of all tuberculosis (TB) cases and about 95% of TB-related deaths. In these areas, more than 75% of TB deaths occur in economically productive age groups, ages 15 to 50 years. [5] Papua New Guinea being a developing nation shares the load of such alarming statistics. [1]

The need for continuous screening for anti-tuberculosis drug is high. Drug discovery processes generally employs traditional, emperical and molecular approaches to drug discovery. All these procedures aim to arrive at a chemical compound that will be effective against TB and without much toxicity. Thus, chemical diversity is needed for such screening.

Natural Products could offer chemical diversity. Plants presents more structural diversity in their secondary metabolites than provided by most available combinatorial approaches based on heterocyclic compounds. Plants and other natural products have a relatively small (>1000Da) compounds. They posses more drug-like properties; i.e., they can be absorbed and metabolized and are three dimensional in structure. Bioactive compounds from plants often occur as part of a family of related molecules so that is possible to isolate a number of homologues and obtain structure activity information.[5, 6]

Since only a small fraction of the world's biodiversity has been tested for biological activity, it can be assumed that plants will continue to offer novel leads for novel therapeutic agents, if they are available for screening.[8] Papua New Guinea's vast flora could provide a range of chemo and bio diversity. Papua New Guinea probably harbours more than five percent of the world's biodiversity within some of the world's most biologically diverse ecosystems. [7]

Based on these facts and figures, this paper aims to validate the hypothesis that plants may provide potential therapeutic targets for activity against TB.

Project Aims

- Screen plant extracts from Papua New Guinea for anti-TB activity using MTT TB Cytotoxicity Assay
- Identify those plants with inhibition greater than 70% inhibition
- Isolation and Compound/structure elucidation of anti-HIV or anti-TB active extracts using HPLC, Mass spectroscopy and NMR.
- Determine the mechanism of action for those plant extracts with ≥70% inhibition.

METHODS

1. Sample Preparations

36 plant parts were collected, dried and macerated.

2. Extraction

15-20g of macerated plants was used. Extraction was done using Soxhlet apparatus with three solvents of different polarity. Hexane, ethyl acetate and methanol were used.

3. Solvent Concentration

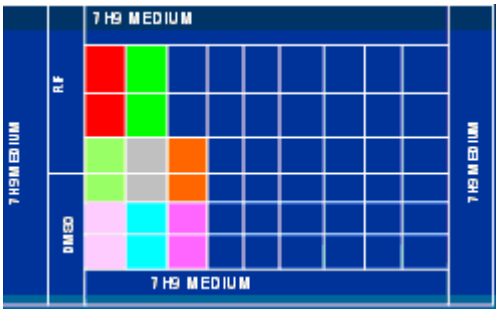
Concentration was done using Rotary Evaporator and Speed Vac. 5mg/ml and 10mgs/ml concentration were made and tested using the assay below.

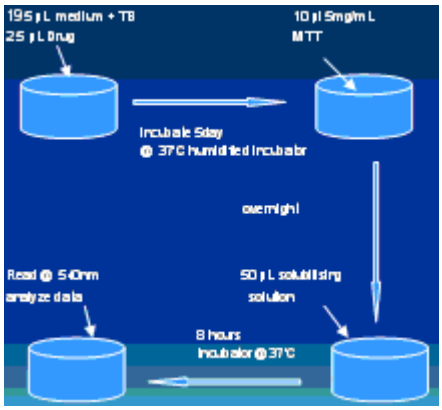
4. MTT Assay

MTT Assay is a Colorimetric method. MTT is a chemical dye, 3-4,5-dimethyl thiazol-2-yl-2,5-diphenyl tetrazolium bromide. It is developed as an alternative method to rapidly and indirectly determine cellular growth based on metabolic activity[9]

The yellow MTT dye is reduced by dehydrogenase in living cells to produce MTT formazin which can be solubilized and read visually or quantified by spectrophotometric measurement at 570nm. TB strain used is H37Ra which in vitro and in vivo studies have shown it to be less virulent and unlikely to be infectious thus a good strain to work with in the laboratory.

The diagram below shows the procedures used. The absorbance of the MTT dye is measured suing a plate reader. The reading is proportional to cell survival thus is used to calculate percentage inhibition.



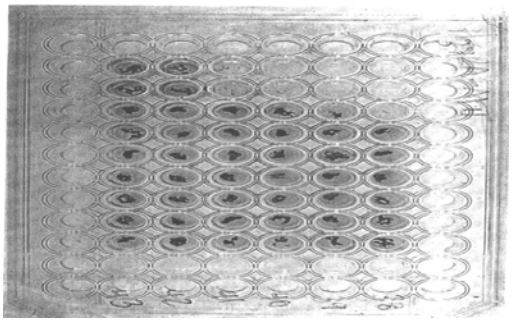


Schematic diagram drawn for the MTT Assay developed by Mashana and his colleges. [9]

Preliminary Results

200 tested extracts for TB has yielded the following results.

- Sample # SU1873- AVERAGE 93%inhibition
- Sample # 76-AVERAGE 92% inhibition
- Sample #118-AVERAGE 98% inhibition
- Sample # 16-AVERAGE 97% inhibition



Photograph showing a 96 well plate with the black spots showing the purple formazin forming from the MTT dye indicating the presence of TB growth while the clear cells showing no TB growth due to Cytotoxic effect of the plant extracts.
Percent of inhibition was made following this formula drawn from an excel sheet.

- AVERAGE CONTROL (AC)
- CELL SURVIVAL PER WELL (CS)
- 100% TB GROWTH DMSO (TG)
- CS-AC = ACTUAL CELL SURVIVAL (ACS)
- ACS/TG * 100= % CELL SURVIVAL
- 100-% CELL SURVIVAL= % INHIBITION
- 90-100% INHIBITION IS A HIT

6. Chemical Isolation and Structure Elucidation.

These active plant extracts will be subjected to further separate chromatographically using HPLC for peak collection. Fractions will be tested again for activity against TB. Fractions with positive results will be chemically elucidated.

7. Isolation and Purification

These compounds will be further isolated and chemical structure will be deduced using NMR, MS and other analytical instruments.

8. Concentration-Dependent Studies

Conconcentration-dependent response will be done once a pure compound is done.

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DETERMINATION OF ANTIMICROBIAL AND ANTITUBERCULOSIS ACTIVITY OF SELECTED MEDICINAL PLANTS

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Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important.[1]

80% of developing countries in the world rely on traditional medicine for their primary health needs, 85% of the traditional medicine involves the use of plants extracts. 74% of today's drugs are derived from plants. PNG is home to many unique plants. Estimated that over 800 medicinal plants have been documented in PNG. Chemical and pharmacological studies are yet to be done. E.g., of chemical compounds derived from plants and used in medicine are; vinblastine, vincristine isolated from *Catharanthus roseus* is now used in cancer chemotherapy[1]. Several traditional medicinal plants of PNG have been investigated to isolate the bioactive compounds. The structure of several compounds isolated from *Ficus septica, Nauclea orientalis.*

In more recent screening by NCI, the family of Guttiferae was interesting. The PNG plants Garcinia achboldiana and hyperium papuana are currently been screened for antiviral activity[2] Tuberculosis in Papua New Guinea is a leading cause of morbidity and mortality and also in many tropical and developing countries of the world. PNG is rank the 8th in the South Pacific regions is cause by MTB (mycobacterium tuberculosis), a contiguous disease that affect almost any part of the body, is mainly the lung infection. Increase in persons infected with MTB in the world and the development of resistance to antibiotics by this microbe and other infectious bacteria, has created the need for new drugs to replace those which have lost effectiveness[7].

The antimicrobial properties of extracts from these medicinal plants will be assayed by two different methods. Plant extracts will be screened for antimicrobial activity using a disc diffusion test. Extracts will also be tested for antituberculosis activity in MTT Assay. Extracts showing antimicrobial or antituberculosis activity will be evaluated by bioautography to correlate the activity with a chemical component. The active ingredient will be isolated by HPLC, and its structure determined by NMR. The data will be analysed to determine if there are correlations between antimicrobial activity, antituberculosis activity and the traditional uses.[8]

Specific Aim and Objectives

The specific aim for the project is to screen and identify drug compounds from traditional medicinal plants with antimicrobial and anti-tuberculosis activity and with the objectives below. It is hope that if the activity can be detected from either one of the plant samples, the study can be pursue further. The main objectives are;

1. To identify and select plants that may have the anti- tuberculosis and antimicrobial activity
2. To make collection of these plants in sufficient quantity for extraction, microbial testing and pharmacological testing.
3. To identify the active compounds and determine structure

MATERIALS AND METHODS

1. Selection and collection of traditional medicinal plants

Plants selected were based on the symptoms for cough, weight loss etc. About 10 medicinal plants have been collected with 16 different samples.

Parts collected were leaves, barks & roots, chopped up and placed in green bags, air dried for one week.
I have labeled the samples with ID number of UPNG-MD001-010.

001-leaves, stem	006-leaves, stem
002-herb	007- herb
003-leaves stem	008-leaves, roots
004-leaves stem	009-herb
005-leaves, bark	010-herb

About seven voucher specimens were collected per samples and a total of 70 voucher specimen were collected, identified and kept in the Herbarium.

2. EXTRACTION

Two methods of extraction were employed. In method A, a known amount of powdered plant materials were extracted with methanol overnight. In method B, a known amount of powdered materials was extracted with diethyl ether; water and organic partitions were collected.

Methanol Extraction-15g of dried plant materials were grounded in a Wiley Mill and soaked in 100ml methanol at room temperature overnight. The extracts were decanted, filtered, and the extracts were dried in the Speed Vac. The weights of the dried samples were than taken and have them refrigerated.

Partition Extraction- 15 g of dried plant material were grounded and placed in 150ml round bottom flask.50ml of distilled water was added to give a homogenous mixture. The mixture was acidified with HCL until the pH reads 2.00. The samples were than swirled in a water bath at 37 Degree Celsius for 20 minutes. Then neutralized with NaOH to give the pH of 7.50ml and was pipetted off into a separating funnel and extracted with 50ml of diethyl ether. The organic and aqueous layer was collected in 100ml containers respectively and refrigerated.

3. ANTIMICROBIAL ASSAY

The extracts were weighed and dried. The calculated amount of solvent will be added to the disk as indicated below.

10mg ext. 10mg/3000ul saturate----- disc 1 with 30ul= 100ug/disc ------disc 2 with 3ul= 10ug/dics

Dissolve samples in appropriate solvent (methanol or water)
And disk used will be around, Disc= 6.5mm saturate with 15.20ul solvent (multiple applications-allow to dry between)

As soon as possible, but not later than 15 minutes, the antibiotic disks must be applied to the agar surface so that the diffusion and growth proceed simultaneously. Apply disks with forceps, and each gently to be sure it adheres.

Incubate bacteria at 37 degree celcius, and fungi at 30 degree celcius (with small dish up) ~ 16 hours. Measure diameter of inhibited zone, including that of disk, to the nearest mm[9]

4. Bioautography Assay

The active extracts will be separated on the TLC plate in $CHCl_3$: CH_3OH:H_2O
(80:18:2).Allow TLC to dry; apply TLC plate to agar plate. Surface streak inoculated with microorganisms that were active against. Incubate overnight 24hrs.Determine which component causes zone of inhibition of Microbial growth.

5. MTT Assay

H37Ra cells are plated onto a 96 well plate in a 200 µL volume and treated with extracts at approximate concentrations of 100 µg/Ml. Plates are wrapped with parafilm and incubated at 37oC for 5 days in a humidified incubator. Add 11 µL MTT (5mg/mL) and incubate overnight. Add DMF: 20% SDS (50:50) to each well to dissolve cells and purple formazan product. Read on plate reader at 570 nm

6. Chemical analysis

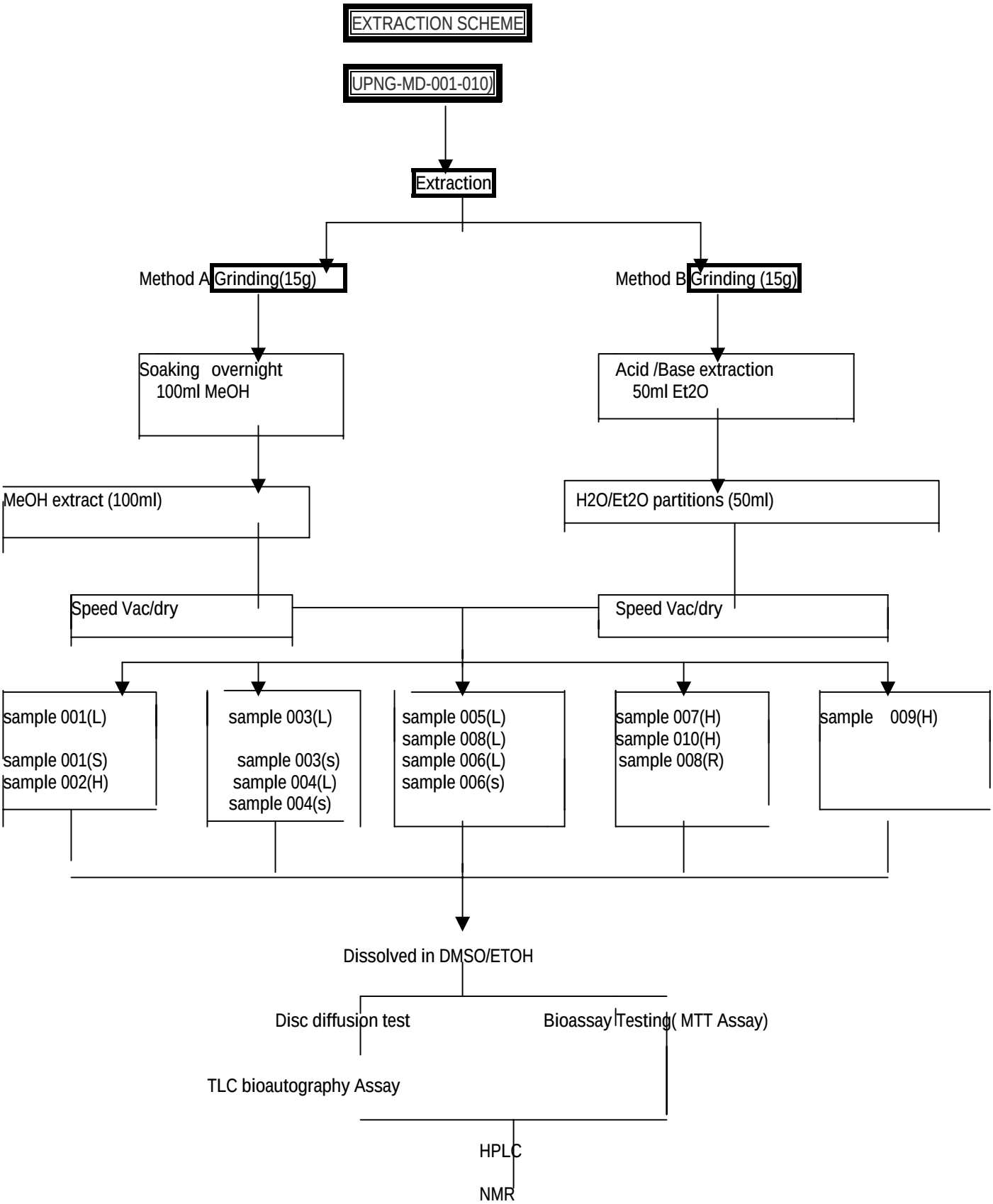
Once the antimicrobial and bioautography assays have been accomplished, the active extracts will be sent to the University of Utah (United States of America) for chemical analysis, using HPLC and NMR, to isolate and identify the active ingredients.

Isolation and Structural Determination Studies

Isolation-methods used for separating compounds such as, PC, TLC, GLC

TLC- is a method for separating all lipids soluble component, lipids, steroids, carotenoid, and chlorophyll

Volatility of higher boiling plant constitute can be enhanced by converting them to esters and or trimethylsilyl ethers so that there are few classes which are completely unsuitable for GLC separation. For preparative work, TLC is carried out on thick layers of absorbent and PC on thick sheets of filter paper. For isolation on an even larger scale than this, it usual to use column chromatography coupled with automatic. The Isolation and
Structural determination studies will be carried out using the HPLC- High Performance Liquid Chromatography and modern NMR-nuclear magnetic resonance.


EXTRACTION SCHEME
UPNG-MD-001-010)
Extraction
Method A Grinding(15g)
Method B Grinding (15g)
Soaking overnight
100ml MeOH
Acid /Base extraction
50ml Et2O
MeOH extract (100ml)
H2O/Et2O partitions (50ml)
Speed Vac/dry
Speed Vac/dry
sample 001(L)
sample 001(S)
sample 002(H)
sample 003(L)
sample 003(s)
sample 004(L)
sample 004(s)
sample 005(L)
sample 008(L)
sample 006(L)
sample 006(s)
sample 007(H)
sample 010(H)
sample 008(R)
sample 009(H)
Dissolved in DMSO/ETOH
Disc diffusion test
Bioassay Testing(MTT Assay)
TLC bioautography Assay
HPLC
NMR

PRELIMINARY RESULTS

1. Documentation

Plants were documented according to the following order
Species: *Musa schizocarpa*
Family: MUSACEAE
Vernacular name: wild banana
Traditional uses: Used in the treatment of stomachache, venereal disease, and strong malaria. In the Sepik, Manus and the Central Province, pregnant women use the leaves as an abortifucent. *Musa paradisieca* is used to treat wounds and cuts[2]. In the Wapenamada, Enga, *Musa* sp. leaves are used on sores. In the Eastern Highlands, the fruit is used to treat dysentery and diarrhea[3]. In Buka Island, new leaf shoots of *M. acuminata* Colla are used to treat cough[3].
Method of preparation: The liquid from the plant is collected in a container, filtered and mixed with a little water for drinking. It is also combined with other plants to make it more effective. Antimicrobial activity: no data available

2. Chemistry

The following columns were eluted using: 25% (F1), 50% (F2), 75% (F3) iPrOH/H2O and 100% (F4) MeOH. Percentages are of total recovered material.

Sample identification	crude 1 ml=		FW %	F1 %	F2 %	F3 %	F4
PNG-20154-006 *taccada*	5.8mg		8.50	0.9	1.5	3.0	3.4
PNG-20154-007 *tiliaceus*	188.1 mg	2.09	0.16	0.71	0.3	0.92	
PNG-20154-009 armocarpoides	1.0 mg			45.00	7.00	19.60	12.70	7.70
PNG-20154-0IO *anisodora*	18 mg			29.50	25.80	1.50	1.20	11.00

Findings:
50% of FW was inorganic salt – 10-20% of organic material distributed in F1-4.

Conclusion

A number of traditional medicinal plants selected, collected and identified, and extracted using various chemical means have shown some variation in the recovery of the materials among the four fractions.
In house screening demonstrated that biological activity generally partitioned into one or two of the fractions F1-4
This method could help researchers to identify which fraction could demonstrate biological activity for bioassay screening by looking at percentage of material recovered.

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ANTIBACTERIAL ACTIVITIES OF PNG MARINE SPONGES

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INTRODUCTION

As the world continues to be infested with so many diseases the search for new medicinal drugs has become so challenging that new thoughts and paradigms have to be developed to combat this age old problem. The resistance of diseases to drugs and the increase in the incidence of the Human Immunodeficiency Virus, other opportunistic diseases and cancer, has prompted scientist to explore nature and its' products in the hope of discovering new drugs.

The field of natural products chemistry has embarked on an effort to find new drugs by concentrating on the isolation of compounds mainly from terrestrial plants. During the past 20 years, however, a core group of marine natural products chemist from several countries, in collaboration with both academic pharmacologists and pharmaceutical industry, has reported a very large number of novel metabolites with useful and sometimes sensational pharmacological properties[1]. About 75 % of the earth's surface is said to be covered by water[2]. This can be directly related to its biological and chemodiversity. Since the study of marine natural products is relatively young, there is a wide array of structurally diverse compounds waiting discovery in the future.

The collection of marine natural products has been enhanced with the use of scuba that has made sampling at 15 to 45 meters possible, thus providing the marine natural products research a largely untapped resource with a range of unique structure classes and novel compounds[3,4].

According to Faulkner (2000), the best sources of pharmacologically active compounds are bacteria (including cyanobacteria), fungi, certain groups of algae, sponges, soft corals and gorgonians, sea hares and nudibranchs, bryozoans, and tunicates. Much of this activity can be attributed to the chemical ecology of the organism and its evolutionary history, with the most primitive organisms being the sources of some of the most active compounds. Since these organisms are sessile and soft bodied and hence lacking physical defences, they have developed chemical defences thus possessing bioactive metabolites. With evolution taking place, it can be supposed that these metabolites have developed other secondary metabolites and perfecting their defence mechanisms and hence their bioactivity. These defence mechanisms, however, cannot be equated directly with potentially biomedical activity though these two can correlate well in reality[1].

Species diversity reaches very high densities on coral reefs, occasionally reaching densities of approximately 1,000 species per square meter, particularly in the Indo-Pacific Ocean, where tropical marine biodiversity reaches its peak[5]. As Papua New Guinea is geographically located in the part of this Indo-Pacific region with 9,980 square kilometres of water [6], it is a good site for high biological and chemical diversity.

This paper reports findings from antibacterial assays of three marine sponges from PNG notably *Phakellia flabellate*, *Cymbastela sp*. and *Epipolasis sp.*

MATERIALS AND METHODS

Marine Organism Collection and Extract Preparation

Approximately 500 grams of marine sponges were collected by hand using SCUBA techniques, from reefs and drop offs at the East Cape area in the Milne Bay Province. The samples were deep frozen on site and transported to Port Moresby over dry ice. Voucher samples of each sample was kept in menthol and sent to the University of Utah, USA for taxonomic identification.

Extracts were prepared by thawing the frozen samples and cutting into very small pieces and homogenised. About 100 grams of each sample was extracted exhaustively with 200 mL of methanol repeatedly for four times. The homogenates were filtered and concentrated on a Buchi R-200 Rotary Evaporator and dried using a Jouan RC1010 centrifuge evaporator to give a gummy residue. About .05 grams of each samples was dissolved in 1 mL of methanol to give a concentration of 4 mg/disc.

TLC Separation

The sample components were separated using aluminium plates and a solvent mixture of methanol and chloroform in a ratio of 1:3 was used as the mobile phase. UV light was used to scan and clarify the separation of compounds. The samples were run through a dragendorff reagent to test for presence alkaloids.

Antibacterial Screening

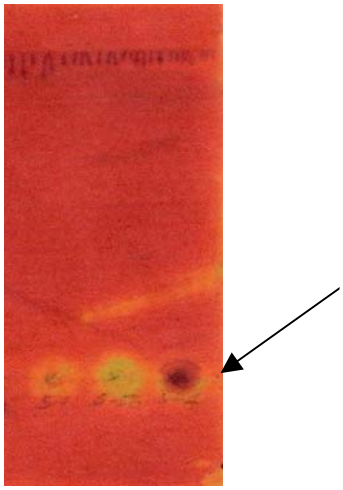
The "Disk paper diffusion" method was performed to test for antibacterial screening. Antibacterial susceptibility test medium Mueller Hinton Agar (Amyl media) was used as media and inoculated with bacteria cultured at the

Microbiology department at the University of Papua New Guinea. Streptomycin was diluted with distilled water to a working concentration of 0.01g/mL and used as standard. The plates were incubated for 24 hours at 37 ^{o}C. Only Serratia marcescens was left at room temperature for the 24 hours.

RESULTS

The following results were attained from the TLC analysis and antibacterial screening performed.

Thin Layer Chromatography



Spots at which extracts had been spotted for separation. From left to right are Phakellia flabellate, Epipolasis and Cymbastella. Cymbastella on the far right shows a dark spot when run through with dragendorrf reagent.

Figure 1: TLC plate

Antibacterial Screening

Table 1: Antimicrobial activity of extractives from *Phakellia flabellata*

Bacteria	Ref [b]	Disc 1	Disc 2	Disc 3	Average
Bacillus Subtilis	**20**	10	10	10	**10**
Staphylococcus Aureus	**16**	10	10	10	**10**
Micrococcus Luteus	**10**	8	8	8	**8**
Escherichia Coli	**12**	6	6	6	**6**
Enterobacter aerogenes	**18**	6	6	6	**6**
Serratia Marscens	**8**	8	10	10	**10**

Values are inhibition zone (mm) and an average of triplicate. The sample is unfractionated methanol extract (conc. 4 mg/disc). The concentration of Streptomycin ([b]) is at 0.01g/mL.

Table 2: Antimicrobial activity of extractives from *Cymbastella sp*

Bacteria	Ref [b]	Disc 1	Disc 2	Disc 3	Average
Bacillus Subtilis	**20**	12	12	10	**12**
Staphylococcus Aureus	**16**	12	12	12	**12**
Micrococcus Luteus	**10**	10	10	10	**10**
Escherichia Coli	**12**	8	8	8	**8**
Enterobacter aerogenes	**18**	10	10	10	**10**
Serratia Marscens	**8**	8	8	8	**8**

Values are inhibition zone (mm) and an average of triplicate. The sample is unfractionated methanol extract (conc. 4 mg/disc). The concentration of Streptomycin ([b]) is at 0.01g/mL.

Table 3: Antimicrobial activity of extractives from *Epipolosis sp*

Bacteria	Ref [b]	Disc 1	Disc 2	Disc 3	Average
Bacillus Subtilis	**20**	8	8	8	**8**
Staphylococcus Aureus	**16**	8	8	8	**8**
Micrococcus Luteus	**10**	6	6	6	**6**
Escherichia Coli	**12**	6	6	6	**6**
Enterobacter aerogenes	**18**	6	6	6	**6**
Serratia Marscens	**8**	0	0	0	**0**

Values are inhibition zone (mm) and an average of triplicate. The sample is unfractionated methanol extract (conc. 4 mg/disc). The concentration of Streptomycin ([b]) is at 0.01g/mL.

Table 4: Results showing the summary of antibacterial activity of tested sponge extracts.

Bacteria	Phakellia flabellata	Epipolasis sp	Cymbastella sp	Ref[b]
Bacillus Subtilis	10	8	12	**20**
Staphylococcus Aureus	10	8	12	**16**
Micrococcus Luteus	8	6	10	**10**
Escherichia Coli	6	6	8	**12**
Enterobacter aerogenes	6	6	10	**18**
Serratia Marscens	10	0	8	**8**

Values are inhibition zone (mm) and an average of triplicate. The samples are unfractionated methanol extract (conc. 4 mg/disc). The concentration of Streptomycin ([b]) is at 0.01g/mL.

DISCUSSION

TLC analysis and test for alkaloids showed that Cymbastella has alkaloids while Phakellia flabellate and Epipolasis showed nil presence of alkaloids. The presence of alkaloid on the plate is indicated by the development of darkened spot when the plate is run through dragendroff reagent. As indicated above, the spots for Phakellia flabellate and Epipolasis are lightly spotted while for cymbastella it is dark.

The entire unfractionated methanol extracts of the samples showed antibacterial activity against six of the bacteria although with varying results. The bioactivity of Cymbastella has some similar results compared to Streptomycin. Cymbastella extracts have equivalent bioactivity with Streptomycin against Micrococcus Luteus and Serratia Marscens with inhibition zones at 10 mm and 8 mm respectively. This bioactivity of cymbastella can be attributed to the alkaloids that have been indicated in picture 1 above.

Phakellia flabellate and Epipolasis both showed some activity against the bacteria but they both have been insignificant compared to the bioactivity of streptomycin. Though it is not mandatory for antibacterial activity, the absence of alkaloids is also a possible reason for less activity encountered in the two extracts.

RECOMMENDATIONS

Although these extracts have shown bioactivity against the said bacterial strains, no particular chemical functional groups can be held responsible for the activities. The test for alkaloids herein described and performed does not indicate the type of alkaloids present. Therefore, it is recommended that further purification and isolated be carried out to purify the active compound, and then to determine its chemical structure.

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MICRONUTRIENT REQUIREMENT IN PERSONS LIVING WITH HIV/AIDS: A REVIEW

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Introduction:

Micronutrient deficiency, now referred to as Vitamin and Mineral Deficiency (VMD), is widespread among women and children in developing countries. "VMDs deprive one billion people world-wide of their intellect, strength and vitality" (1). Individuals with multiple micronutrient deficiencies are in a state of micronutrient starvation. In other words, they suffer from the "Hidden Hunger" that secretly suppresses their immune response, increasing the risk of developing infectious diseases (2). The idea that functions of the organs (Thymus, Spleen, Lymph nodes) of the Immune system are compromised in the malnourished individual cannot be overemphasized. There are contradictions and controversies in published scientific data on the effect of micronutrient deficiency on the immune system during infection. The situation becomes even more complex when it involves conditions such as Human Immunodeficiency Virus (HIV) infection. The role of micronutrients in infection is difficult to study with the usual scientific research methods because of complex interactions between the Gastrointestinal tract, Immune system, Viral replication and various micronutrients (3, 4). According to Peabody (3), the metabolism of micronutrients is altered during the acute phase response, making it difficult to accurately assess deficiency.

Most research studies (2,3,4) on micronutrients deal with sub-clinical deficiencies, meaning that there may or may not be apparent physical symptoms of deficiency. It must be noted, however, that low levels of serum/plasma micronutrients in an individual or in a population could mean that there actually is deficiency with more generalized malnutrition (5). It could also reflect a change in the metabolism of the micronutrient (2,3,5), indicate a temporary response to an infection (4), a marker of disease progression (the result of an interaction between a drug and the micronutrient), or even a laboratory error (2,3,5). These are some of the variables that have resulted in the lack of consensus in the scientific community on the issue of micronutrients. Lack of consensus, in turn, is at the root of the general reluctance to make recommendations for specific micronutrient supplements. For example, most of the WHO approved recommendations for daily micronutrient intake is developed based on deficiency prevention in the "healthy" population (1,4). There is still no WHO approved acceptable recommended daily micronutrient intake for Persons Living With HIV/AIDS (PLWHA). This is because the effect of micronutrients on HIV/AIDS and the long-term consequences of taking high doses of various micronutrients are still not fully understood (3,6). However, it is common knowledge that PLWHA are always requested to use micronutrient supplements, because convincing scientific research data indicate that Micronutrient deficiencies are common in PLWHA (6,7,8,9,10,11,12). The reasons for micronutrient deficiencies in PLWHA are manifold – these may include malabsorption in the GIT of the patient, diarrhoea and also depletion caused by the virus (6,13). Most of the published scientific data on the effect of micronutrients on PLWHA were obtained from studies that pre-date the use of Highly Active Anti-Retroviral Therapy (HAART). Only a few of these studies have been conducted on PLWHA using HAART (2 – 13).

The aim of this brief review, which is mainly focused on the pre-HAART studies, is to stimulate discussion on the significance of micronutrients and at the same time to advocate for adequate micronutrient supplementation to become part of the appropriate therapy for PLWHA. Current data (13,14) indicates that HIV infection involves a progressive immune dysfunction and loss of CD4 T-cells leading to opportunistic infection, wasting syndrome, malignancies, and other metabolic disorders. Some researches have proposed that the apoptosis of CD4 cells contributing to HIV progression does not result solely from HIV infection, but also from antioxidant imbalances in the human host (13,14,15). Another group of researchers (16) reported that cellular apoptosis, the main cause of lymphocyte depletion in HIV, is linked to the Red-Ox status of Glutathione. They also indicated that many immunological functions in HIV disease are dependent on adequate amounts of reduced Glutathione. The evidence in support of these views was based on the following (14,15): Activation of latent HIV state can be stimulated in the presence of Reactive Oxygen Species (ROS) through the stimulation of O_2-responsive Transcription factors, specifically Nuclear Factor Kappa B (NF-kB), which is a protein found in T-cells. Stimulation of Nuclear Factor Kappa B is required to induce HIV replication in the infected T-lymphocyte. A reduction in the cellular level of ROS inhibits the stimulation of NF-kB and thus prevents the replication of HIV in infected T-lymphocytes. These authors (14,15) further proposed that by restoring the correct cellular redox balance through adequate availability of Anti-oxidants the number of ROS could be reduced.

Micronutrient deficiencies have long been reported to be a common feature in PLWHA both in the early and late stages of the disease (5,6,8,9). However, the findings by Tomaka et al (17) that Selenium and Beta-carotene are two of the major micronutrients that are deficient in PLWHA provide a logical explanation for their role as antioxidants. They reported that the prevalence of micronutrient deficiency in all the 129 patients with stratified T-cell counts studied were similar (17). Among the three subgroups studied (CD4 > 500, CD4 200 – 500, CD4 < 200), each had similar occurrence of micronutrient deficiencies: 38%, 41% and 42% respectively (17). Selenium and Beta-carotene deficiencies were prominent in the three subgroups studied. Selenium as an Anti-oxidant in the host: The major anti-oxidant function of Selenium is via the antioxidant metallo-enzyme Glutathione Peroxidase, of which selenium is an essential structural component. Selenium plays a significant role in the maintenance of humoral and cell-mediated immunity (18,12). The oxidative damage to tissues is reduced when Glutathione Peroxidase catalyses the removal of Pro-oxidants, such as Peroxides. The role of selenium as anti-oxidant in HIV/AIDS appears to be related to both (a) direct immune modulation and (b) inhibition of Cytokine and NF-kB activation, which suppresses HIV replication (9,13,19,20). Selenium also acts as a modulator of Lipo-peroxide-related activity. Low Selenium levels in blood correlate with low Glutathione Peroxidase activity in PLWHA (13).

Selenium also plays an important role in the production of Thyroid hormones. There are two enzyme systems involved: The liver and kidneys have a low affinity, high capacity enzyme system (Iodothyronine Deiodinase) that regulates circulating Tri-iodothyronine (T3) levels by catalysing the conversion of Thyroxine (T4) to T3. Iodothyronine Deiodinase is a metallo-enzyme that utilizes Selenium (it contains Seleno-Cysteine).

The second enzyme system (5-Deiodinase) does not require Selenium: it catalyses the conversion of T4 to reverse T3 (rT3). Therefore, deficiency of the trace element Selenium can result in a decrease in the conversion of T4 to T3, thus causing an increase in the conversion of T4 to rT3, by the enzyme 5-Deiodinase that does not contain the amino acid Seleno-Cysteine (19,20). Viral Seleno-proteins and HIV: Taylor et al (20) documented the existence of a Selenium-based homolog of Glutathione Peroxidase produced by the HIV virus. The HIV gene is capable of accelerating the depletion of selenium from HIV-infected lymphocytes, allowing the virus to replicate at the expense of the CD4 cells (20). The Seleno-proteins in viruses tend to regulate viral growth, both by inhibition and promotion, depending on the redox environment and the concentration of selenium in the host (20). Patients who are co-infected with Hepatitis C and HIV-1 have lower levels of Selenium than HIV patients at the same stage of the disease but who are negative for Hepatitis C (11).

Selenium deficiency, more than any other micronutrient deficiency, has been documented to correlate with high progression and mortality rates in HIV/AIDS (13,21). Selenium level in host plasma appears to decline consistently as HIV progresses. Selenium deficiency correlates with immuno-competence, and can predict mortality (21). Selenium deficiency is associated with immune dysfunction, impaired resistance to microbial and viral infections, inadequate phagocytosis and antibody production, impaired lymphokine production, natural killer cell cyto-destruction, and with decreased CD4 cell counts (13,18,19). Trials of selenium as an antioxidant in PLWHA have been inconsistent, due to a wide variety of treatment protocols, but they still offer significant promise as adjunctive treatment in HIV/AIDS (13,18,19). A number of supplementation studies indicate improvement in the selenium status in PLWHA, accompanied by slower HIV replication, and a considerable reduction in the production of cytokines that cause inflammation (2,10). The recommended daily intake (RDI) of selenium ranges from 70 μg to 200 μg (22). The RDI represents the minimum amount required to prevent a clear deficiency in a healthy sedentary adult population without chronic diseases. The Upper Tolerable Limit (UTL) set by the USA National Academy of Sciences for Selenium is 400 μg (22). Best dietary sources of selenium are Brazil nuts, seafood, liver, meat and grains (22).

Zinc is an important trace element with antioxidant properties. It protects cells from the damaging effects of ROS generated during immune activation (4). Zinc is important for the production and activity of T-lymphocytes and B-lymphocytes (12). Zinc is also a co-factor in a vast number of metabolic reactions. Data on Zinc requirement for PLWHA are contradictory. The Zinc status of PLWHA has been associated with both positive and adverse clinical outcomes (23,24). PLWHA that consume high amounts of Zinc (over 20mg per day) are twice as likely to progress faster to full-blown AIDS than those with a lower Zinc intake (23,24). Baum et al (25) reported that Zinc supplementation improved the CD4 count in PLWHA. In a very recently concluded study, Fawzi et al (26) examined the effects of Zinc (25mg Zn given orally daily) supplements on HIV-1-infected pregnant women in Tanzania. All the women in the experimental and placebo groups received iron, folic acid, and multivitamin supplements. The authors (26) reported no beneficial effect of Zinc supplementation on adverse pregnancy outcomes and, moreover, its possible negative effect on Hb concentrations, and concluded, therefore, that no compelling evidence exists to support the addition of Zinc to prenatal supplements intended for pregnant HIV-1-infected women. They also stated that Zinc had no effect on CD4+, CD8+, or CD3+ cell counts during the following-up period after the study (26).

Beta-Carotene and Vitamin A: Beta-carotene is an anti-oxidant and plays a major role in trapping Peroxy free radicals in tissues. The ability of beta-carotene to act as anti-oxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure (9,13). As an antioxidant, beta-carotene appears to support enzymatic defence systems involved in minimizing oxidative damage (9,13). Beta-carotene inhibits both the production of Cytokines and the activation of NF-kB, resulting in inhibition of HIV replication (9,13,19,20). Beta-carotene has been shown to act directly as an immuno-modulator by increasing natural killer cell function and improving CD4 count (7,13,27). Beta-carotene deficiency is common in all stages of HIV/AIDS and may signal malabsorption (27). Supplementation has been shown to affect specific T-lymphocyte cells and to decrease markers of Lipo peroxides; various measures of oxidative stress were also improved (2,13). Data from controlled supplementation trials did not show any significant differences in the WBC count, CD4+ count, or other measures of immune function in PLWHA taking beta-carotene or placebo (8,9). Beta –carotene is the precursor for Vitamin A: Research reports on Vitamin A and the progression of HIV are conflicting. Vitamin A intake was positively associated with the CD4+ count at baseline and with a slower progression to AIDS (28). Data also indicate that moderate Vitamin A intake was associated with a reduced risk of HIV progression and death, but that high intake was not protective (23,28). Data from randomized trials indicate that Vitamin A supplementation slows progression of HIV disease (12). Fawzi (26) reported that daily Vitamin A supplementation had no significant effect on CD4+, CD8+ and CD3+ cell counts during pregnancy.

Vitamin A deficiency has been shown to be an independent predictor of survival in PLWHA (24). High intake of Vitamin A or Beta-carotene have been linked to lower risk of developing AIDS and lower death rates among PLWHA, with an important exception: PLWHA with the highest intake of either micronutrient (over 10,000 IU per day of beta-carotene, or more than 20,000 IU per day of Vitamin A) did worse than those who took less (23,24). The recommended daily intake for Vitamin A and Beta-carotene ranges from 5,000 to 10,000 IU in both cases (22). The Upper Tolerance Limit is 10,000 IU (22). Excess intake of Vitamin A is toxic to the liver and it promotes formation of free radicals in tissues (22). Vitamin B_{12} (Cobalamin) is an important component in the biosynthesis of Purine, Pyrimidine and nucleic acids. A glycoprotein (Intrinsic Factor) is required for the absorption of Vitamin B_{12} in the small intestine. Vitamin B_{12} deficiency may be due to malabsorption and diarrhoea in PLWHA (24,25). Vitamin B_{12} deficiency (< 120pmol/L) has been linked to lower CD4+ counts and more rapid development of AIDS (24,25). Improving the plasma Vitamin B_{12} level (to > 120pmol/L) by supplementation increases both T-cell counts and natural killer cell activity in PLWHA (23). According to Garcia-Die et al (29), the low serum Vitamin B_{12} in HIV-infected

patients should not be considered as deficiency *per se* if it is not accompanied by low RBC Folate concentration in the patient. Ramacha et al (30) reported low concentration of Vitamin B_{12} and RBC Folate in 20% and 10% of HIV-infected patients, respectively. In a recent study, Remacha et al (30) reported that PLWHA receiving HAART have lower prevalence of reduced Vitamin B_{12} and higher concentrations of Hb, leukocytes, CD4, and CD8 compared to those in the non-HAART group.

Available data indicate that in PLWHA higher intake of the antioxidant vitamins (Vitamin C and E) are associated with improved CD4+ cell counts and a slower disease progression (12,28). Supplementation with both Vitamins C and E at the same time showed a significant reduction in HIV load (12,28).

Alpha-Lipoic Acid (ALA): Another antioxidant studied in PLWHA is Alpha-Lipoic acid (ALA). It is a water- and fat-soluble vitamin-like substance that can cross most cell membranes. It is the most powerful antagonist known of a human cellular protein called Nuclear Factor Kappa B, which has to be activated in order for HIV to transcribe its genes (2). Some pre-HAART studies have shown a direct inhibition of HIV replication in patients given ALA (2). ALA appears to be able to regenerate Vitamins C and E, which can further enhance the overall antioxidant status in an individual (2).

Other essential nutrients: Omega-3 (ω-3 or n-3) fatty acids, which are found mainly in fish oils, enhance immune function (2,22). Two examples of Omega-3 fatty acids are Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Both fatty acids decrease inflammation by modulating and influencing the Cytokine production of T-cells (22). Omega-3 fatty acids reduce the ability of some immune cells to react to infectious organisms; DHA can decrease the activity of natural killer cells (22).

Micronutrient complex (Multivitamin and Trace elements): Single micronutrient deficiency usually does not occur in humans. Thus, supplementation using adequately prepared micronutrient complexes has always been the logical choice for maintaining an appropriate micronutrient balance in the body. Abrams et al (28) reported an increase in the CD4+ cell count and a 31% reduction in HIV disease progression in PLWHA receiving daily multivitamin and mineral supplements. Prenatal multivitamin supplementation (excluding Vitamin A) significantly improved CD4+, CD8+, and CD3+ cell counts in HIV-infected pregnant women in randomised trials in Tanzania (26). In general, observational data indicate that multivitamin and mineral supplementation slows the clinical and immunologic progression of HIV-1 disease (2,5,9,28).

In conclusion “We are what we eat”. Understanding the intricate relationship existing between the need for improved immune status and micronutrient requirements in PLWHA is an area that demands greater research resources, especially in the new era of HAART. Maintaining optimal nutritional status and adequate vitamin and mineral stores in the body are the most essential and effective ways of mounting an effective immune response to opportunistic infections, especially for PLWHA. One can achieve this by striving to consume adequate amounts of well-balanced and varied foods including fruits and vegetables and supported with a well thought out and planned micronutrient (Vitamins and Trace elements) supplementation.

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HOMEOSTASIS – THE KEY TO SOCIETAL STABILITY

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Physiology is the branch of natural science that studies the mechanisms of body function. Its goal in teaching and research is to understand the integrative function of living organisms from the level of molecules, to cells, to organs, to the whole organism. The focus concept is *homeostasis* –maintenance of internal stability.

Homeostasis was coined by Claude Bernard (1813 – 1878). He defined it as follows: All vital mechanisms, varied as they are, have only one objects, that of preserving constant the conditions of life in the internal environment.

Walter Cannon, Chairman of Physiology at Harvard in the 1930s, expanded the concept to include:

- ✓ stability of any physiological variable, eg. Blood pressure.
- ✓ stability of a whole organism & societies of organisms

Within life science, homeostasis therefore includes both:

- ✓ Physiological, and
- ✓ Behavioural facets.

Action and interaction of physiological variables occurs by communication:

- within and
- among all levels of organization in living systems

Organizational levels of Living Systems are:

- Societies
- Organisms
- Organ Systems
- Organs
- Tissues
- Cells
- Molecules

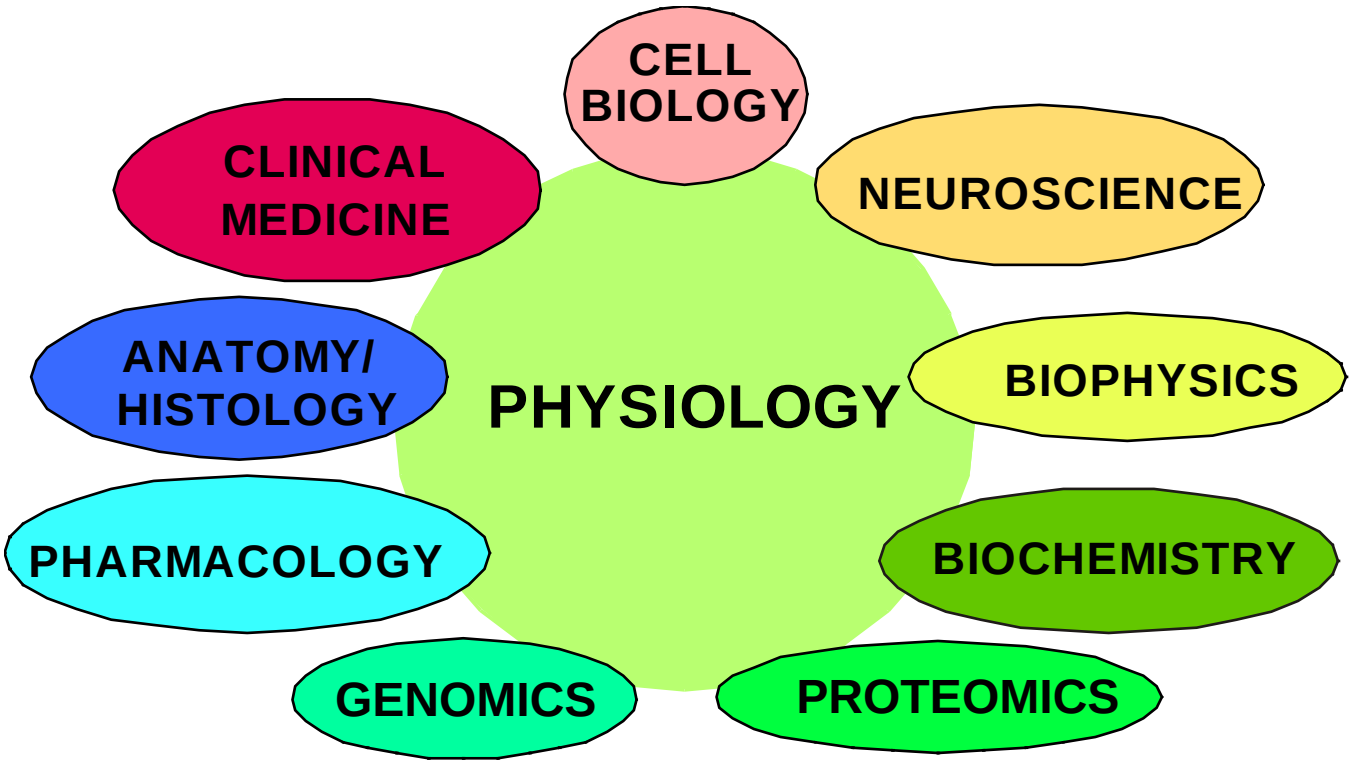
Understanding the importance of the concept of homeostasis, it plays a central role in the curriculum of the following disciplines:

- Medicine,
- Nursing,
- Veterinary medicine,
- Pharmacy,
- Dentistry,
- Occupational Therapy,
- Speech Therapy,
- Physiotherapy,
- Optometry,
- other health sciences disciplines

Principles of homeostasis are important in medical & health education as new drugs are introduced, novel diagnostic and interventional techniques are developed, and better understanding is obtained of how genome alters function. In clinical practice, an understanding of homeostasis is an integral part of the evaluation of the clinical case. The symptoms, signs, and investigation findings reveal how normal physical (homeostasis) or psychological function is disturbed. Evaluation of a clinical case also requires an ability to integrate this understanding of function, be it normal or abnormal, with other medical sciences.

The figure below depicts the relationship between homeostasis/physiology and the other biomedical sciences.

Defining scope/relations of physiology[2]



Teaching Homeostasis Across Disciplines

The above nomenclature looks narrow in the context of disseminating information to a broader audience in the prevailing world. In the article of Snow, C. 'The two Cultures: And a Second Look' published in Cambridge Univ. Press, UK in 1964. He warned about the emergence of two educated groups in the world that cannot communicate with each other. These are:

- ✓ Scientists, and
- ✓ literary intellectuals

This trend is occurring at a time science is determining much of our destiny – whether we live or die The pretence of a common culture has been lost and this leads to interpreting: the past wrongly, to misjudge the present, and to deny our hopes of the future.

This is serious for our creative, intellectual and, above all, our normal life. Scientists can give bad advice and decision-makers can't know if it is good or bad.

Science should be of great relevance not only to those who would become scientists but even more to those who will not. Education that deprives non-science students of an awareness of what science is also short changes would-be scientists. To correct this anomaly, it is recommended that:

- Homeostasis be taught to all non science majors in the Universities, and
- Health Sciences students should be taught the influences of social environment on body function.

Central principle common to both health and social sciences is homeostasis Walter Cannon in 1932 book 'The Wisdom of the Body" stated that homeostasis is both a physiological and sociological phenomenon. The relationships of biological & social homeostasis are in the book's eepilogue pp 287-305. Earlier, Herbert Spencer, compared society to human body in his writings in the 1800s.

Physiologists use the principles of negative feedback to describe homeostasis. Sociologists use the terms - stress-coping-adaptation to describe the stimulus-response-feedback characteristics of negative feedback (A.G. Peirce. Stress coping and adaptation. Nursing

Leadership Forum 1: 84-89, 1995). The author drew parallel between the phenomena of stress, coping, adaptation & complexity as they pertain to human living in a social environment.

The relationship between physiological and social homeostasis was further advanced in the articles of Lazarus and Folkman, 'The stress concept in the life sciences' (in: Stress Appraisal and coping, R.S. Lazarus and S.Folkman eds, 1984, pp 1-21), 'The concept of coping', (1984, pp 117-140). These authors are the leading authorities on psychological processes of stress & coping. They provide a good background and definition of the key concepts, e.g., transactional stress. Recently they have shifted their emphasis towards positive outcomes of the stress- coping process. (Am. Psychol. June, 2000, pp 647-654).

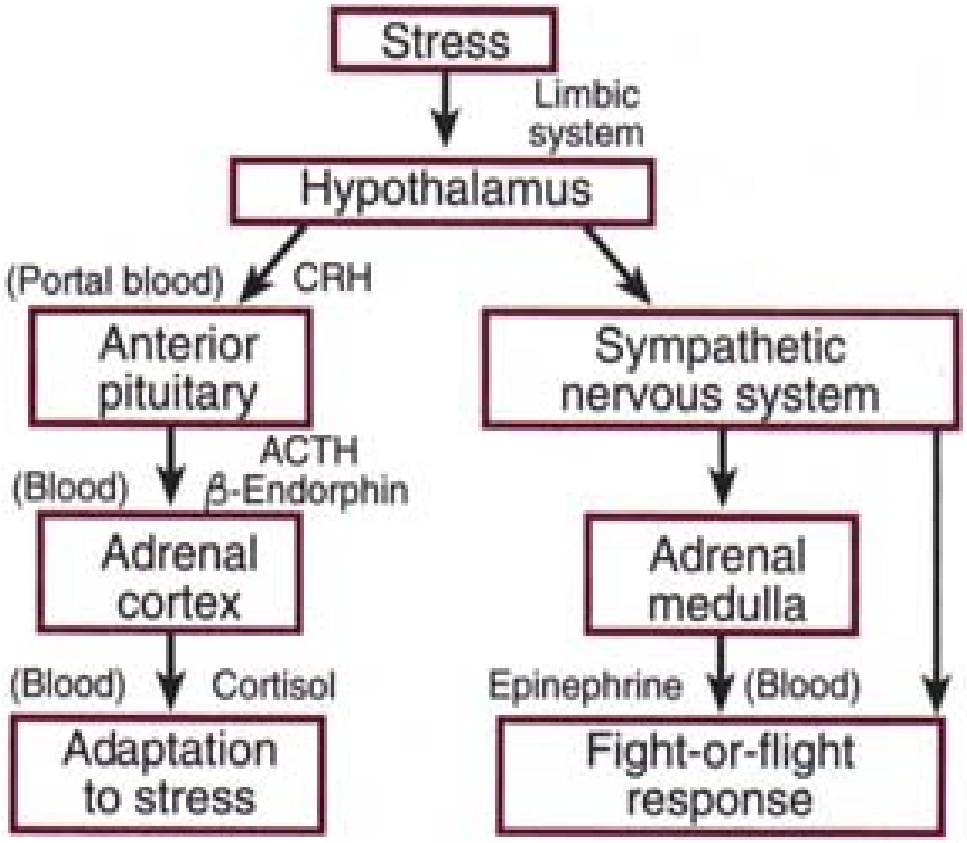
Some examples of how the social environment affects body function could be found in the mechanisms of the following processes:

- ❑ the fight or flight response;
- ❑ hypertension;
- ❑ asthma;
- ❑ type II diabetes;
- ❑ aging;
- ❑ cardiopulmonary resuscitation;
- ❑ sex practices;
- ❑ alternative & complementary medicine etc.
- ❑ the relationship between nutrition and disease;
- ❑ depression
- ❑ eating disorder
- ❑ abuse

The fight or flight response - Physiological changes that occur in response to a perceived threat, including

- secretion of glucose,
- endorphins, and
- hormones as well as,
- elevation of:

    - ✓ heart rate,
    - ✓ metabolism,
    - ✓ blood pressure,
    - ✓ palpitations,
    - ✓ breathing and muscle tension



Hypertension - ↑BP in excess of normal range for person's age & sex
Causes of secondary hypertension are: Cardiovascular hypertension – chronically elevated TPR- atherosclerosis; Renal Hypertension – Occlusion of renal artery or disease of Kidney; Endocrine hypertension – pheochromocytoma and Conn's disease; and Neurogenic Hypertension

Primary hypertension is caused by a variety of unknown causes such as: Genetics; Obesity; Stress; Smoking and Dietary habits.

Challenges in teaching physiology

Understanding the expanding scope of physiology
2. Managing the volume, complexity of knowledge
3. Staying up-to-date with best educational practice
4. Using information technology effectively
5. Encouraging group work and practical skills
6. Supporting physiology in integrated programs
7. Accessing professional educational development for staff, on-line and face-to-face

References

1. Walter Cannon (1932) 'The Wisdom of the Body. Havard Univ Press
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PREPARATION OF A TOXIC SUBSTANCES CURRICULUM FOR TRAINING OF COMMUNITY HEALTH WORKERS (CHW) FOR THE PNG ARTISANAL AND SMALL SCALE MINING PROJECT

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Introduction

The Wau Ecology Institute (WEI) is working with the Department of Mining (DoM) on the PNG Artisanal and Small Scale Mining Project (the Project) which is funded by a grant from the Japan Social Development Fund (JSDF). The grant is administered through the International Bank for Reconstruction and Development - World Bank. The PNG Government supports and is committed to the objectives of the Project. The main purpose of the Project is to improve rural incomes and to mitigate or eliminate the negative, social and cultural effects of small-scale mining on communities. Amalgamation, the process of recovering gold with mercury (1), is extensively used by small scale miners throughout the country and the long term effects of unsafe handling and usage of mercury on the health of miners and the mining communities is not well understood by the miners and health professionals working in these communities. DoM has produced education booklets and videos on safe methods of mercury usage and has conducted outreach programs in some small-scale mining communities (2). However, health professionals remain poorly informed of the serious health consequences of the long-term use on mercury and other toxic substances and have little understanding of diagnoses and treatments. The present curriculum for Community Health Workers and other health professionals does not include any units on toxic substances injurious to health (3).

Objective

The object of this project is to educate all health professionals on the dangers of toxic substances, especially mercury, cyanide, petrol, kerosene, benzene, insecticides and detergents and their effect on health if misused, the symptoms and diagnosis of poisoning, the treatment and safe handling practices by developing a curriculum unit for inclusion in the eduction and training courses for health professionals and Trainees. Over the long term, this should result in better treatment and reduced health risks for small-scale mining communities.

A: Proposed Toxic Substance Curriculum

Exploratory work including interviews and consultation with the Human Resource Department (NDOH) revealed that the CHW and even other related program under the old College of Allied Health (NDOH) structure were ad hoc in nature (4). There was no coverage of substance toxicity specifically relating to mercury, cyanide, kerosene, petrol, benzene, insecticides and detergent in the curriculum. Attempts at properly structured programs were developed for this category of Health workers only after several Universities started offering Health Sciences programs such as those at the Divine Word University's (DWU) Faculty of Health Science, and Pacific Adventist University's (PAU) School of Health Sciences.

B: Competency-based standards and Learning Outcomes

In developing the curriculum, the author was mindful of the criteria developed by NDOH, which adopts the competency-based standards for health workers curricula in PNG (5). In light of this, a number of learning outcomes were drawn up to address the competencies under two (2) domains that espoused substance toxicity; (1) Health and Safety, and (2) Disease Control (Non-communicable diseases). The overview of possible learning outcomes is, but not limited to, the following areas. These topic discussions will enable Health workers and students to fully appreciate the relevance and importance of substance toxicity as an occupational hazard associated with artisanal and small scale mining operations in PNG. Thus, it is envisaged that by the end of the course the CHW should be able to:

(1) Explain what mercury, cyanide and hydrocarbons are?
(2) State the consequences of careless handling or abuse of these different toxic substances?
(3) State and explain what enzymes and proteins are; their importance, and the role they play in biological systems
(4) State which relevant enzymes and proteins are specifically affected by each of the toxic substances
(5) The different types of mercury compounds, and explain their associated risk to health
(6) Explain the pathophysiology of mercury, cyanide, and hydrocarbon toxicity
(7) Illustrate how to apply First aid treatment to casualties of mercury, cyanide and hydrocarbon intoxication
(8) Explain and demonstrate how can you prevent toxicity in the first place?
(9) How to practice proper safety procedures
(10) Recognize and explain the different pathologies associated with poisoning by the different toxic substances
(11) Explain how to diagnose toxicity associated by the different toxic substances
(12) Explain the different and appropriate antidotes for toxicity caused by mercury, cyanide and hydrocarbon
(13) Perform the relevant tests and initiate the appropriate treatment for the relevant types of substance toxicity
(14) Suspect the appropriate and relevant substance poisoning from the clinical features presented by a contaminated patient

These learning outcomes, though not exhaustive, would equip CHWs with the necessary knowledge to make more accurate diagnosis of possible toxicity cases. At present such cases often escape accurate diagnosis due to lack of the necessary skill and knowledge to recognise the typical sign and symptoms of substance toxicity.

D: Brief Structure overview of the curriculum

The proposed structure of the curriculum is shown below in the summary outline (I). It involves specific lecture topics, possible practical classes, as well as a list of possible learning outcomes that must be achieved at the end of each lecture topic. These learning activities are deemed essential and relevant insofar as mercury, cyanide and hydrocarbon toxicity discussions are concerned. The curriculum is made up of three (3) units: Unit 1 deals with all relevant background information about mercury, and mercury toxicity, Unit 2 outlines

those involving cyanide, and Unit 3 details those with hydrocarbons, with particular emphasis on kerosene, petrol, benzene, detergents and insecticides being the most prevalent hydrocarbons encountered in alluvial mining communities in PNG.

Each unit begins with an overview and basics of the relevant lecture topic proposed, bearing in mind the target audience who are Community Health Workers (CHWs). For instance in Unit 1, mercury is discussed in an overview format rather than in detail. This is followed by the various relevant areas that forms the body of discussion involving mercury toxicity, which include; the basic biochemical basis and mechanisms of toxicity (Clinical manifestations), pathophysiology, overview of enzyme and proteins in tissues that are targeted by mercury, recognition of toxicity and explanation of the signs and symptoms, the clinical presentations, management and preventative care issues (Safety), and concluding each Unit with first aid treatment protocols and procedures.

E: Proposed levels of Unit Presentations

The author proposes three (3) levels of presentation of the materials contained and detailed in the course outline. It is firmly believed that in order for health-related issues and information to penetrate the various tiers and cadre of health workers, appropriate delivery mechanisms must be employed. Hence, the curriculum on substance toxicity here will be tailored into the levels of: (1) Certificate, (2) Diploma, and (3), Degree level. In essence, the target or intended audience will determine the depth of materials and details that will be covered and delivered.

F: Proposed number of lecture hours

The number of hours proposed to be spent for the seminar series maybe as follows;

(a) three (3) hours total allocated to Health Science programs and a 3 one (1) practical class each to cover each topic overview of mercury, cyanide and kerosene or petrol
(b) Twenty (21) hours allocated to MBBS program, i.e. 6 hours per week on (PBL program) Patient & Community (P&C) problems for each of the three (3) topics plus 3 hours seminar or lecture
(c) 3 hour (seminar) plus 3 one hour (practical) each for PAU and its affiliates
(d) 3 hour (seminar) plus 3 one hour (practical) each for DWU and its affiliates
(e) 1-2 hour (seminar) each in the mining townships and secondary schools of PNG

G: Practical sessions

This is an integral part of the curriculum as it arms the health workers with the necessary practical experience and skill to deal with eventualities involving the toxic substances under discussion. First Aid, Site visitations, Safety, Exhibits, models and pictorial pathways form a major component of the practical sessions, in addition to basic analytical and determination techniques. Such exercises will augment the theoretical background that they receive and enables the students to appreciate fully the importance and relevance in discussing these substances. The curriculum draft outlines some of the practical exercises that can be carried out; emphasis here is on First Aid treatment and patient management following intoxication.

H: Seminar Questionnaires

Questionnaires have been designed to gauze the views of the participants in the proposed seminar series to be conducted at Wau and Madang. The School of Medicine and Health Science, UPNG will be asked to review the entire program. These questionnaires will assist in restructuring the content of the proposed curriculum. Such an exercise will be important in tailoring the content of the materials to suit the intended audience. 2 separate questionnaires will be asked of the audience, a pre- seminar and a post-seminar. This will give some indications of the level of understand, exposure or awareness of CHW before and after the seminars.

I: Toxic Substances Curriculum

UNIT 1: MERCURY TOXICITY PROPOSED COURSE OUTLINE

WEEK	Lecture topics	Learning outcomes	Practical Exercises
1	Overview: Chemistry of Mercury: Potential sources-emphasis in industrial usage Overview of elemental mercury, Inorganic mercury, and Organic mercury	At the end of the topic you are expected to know: • What is mercury • What mercury is used for • What are the three classes of mercury • What is the difference between these classes of mercury	Table of Elements: Position of Mercury, properties, characteristic, etc.
2	Introduction of Macromolecules: Introduction to proteins and enzymes	At the end of the topic you are expected to know: • What are enzyme • What are proteins and macromolecules • What are the properties of enzymes • What role enzymes play in biological systems	Pictorial and model examples of proteins, macromolecules and enzymes

3	Pathophysiology: Toxic effects of Mercury, and its derivatives in tissues, Mechanism of action of Mercurial toxicity: Acute and Chronic toxicity, Target tissues of toxicity	At the end of the topic you are expected to know: • What is the biochemical basis of mercury toxicity • What are the biological targets for mercury toxicity • What is the difference between acute and chronic mercury toxicity • What are the pathologies associated with mercury poisoning	Class demonstration of Sulphur- containing proteins attack by Mercury and Mercurial derivatives
4	Clinical features of toxicity, Laboratory assessment and findings of possible toxicity,	At the end of the topic you are expected to know: • What are the signs and symptoms of toxicity • What are the typical features and presentations of mercury poisoning • What are the tests used to assess mercury toxicity • What are the biological fluids used to assay possible mercury toxicity • How do you interpret mercury results	Pictorial exhibits of Toxicity, Recognition of potential Signs and Symptoms of Mercury toxicity and exposure
5	Biological detoxification of Mercury: Enzymes and Proteins that are involved	At the end of the topic you are expected to know: • What are the enzymes needed to detoxify mercury toxicity • What are the proteins and macromolecules that play a role in detoxification • What are the location of these enzymes, protein and macromolecules	Tutorial outlining biological systems and processes available in tissues to perform Mercury detoxification
6	Preventative measures against Mercury toxicity: Demonstration of use of proper safety gears, and measures	At the end of the topic you are expected to know: • What are the safety procedures used to avoid contamination • The different safety gears that are available to use when handling mercury • How to prevent contamination in the first place	Exhibits of basic safety gears e.g. safety gloves, nose muffs, eye wear, distil water (eye wash bottles) Safety instructions and procedures
7	Introduction to First Aid: First Aid course on Mercury toxicity, attention, and application, Treatment of Mercury toxicity: Overview of currently available medications, Description of current emergency therapy and treatment regime against Mercury toxicity	At the end of the topic you are expected to know: • Basic general first aid procedures • How to apply first aid during a possible mercury poisoning incident • What are the current antidotes to treat mercury poisoning	First Aid exercises to demonstrate alleviation of toxicity, Emergency treatment at scene of accident (Toxicity)
8	Management of Mercury toxicity	At the end of the topic you are expected to know: • How to manage a casualty of mercury poisoning • What the management procedures and regimes are • What are the treatment and antidotes used in the management of casualties	Demonstration /Review of procedures
9	Field trips to Prospecting sites: Education awareness of Mercury toxicity, Presentation in English and accurate translation in Pidgin	At the end of the trip you are expected to: • Appreciate the importance of mercury as an occupational hazard • Able to relate and interpret basic instructions in pidgin	Field Trip and awareness exercises to educate local prospectors,

		• Know correct techniques and postures that will minimize/prevent occupational injuries	
10	BASIC QUIZZ ON	MERCURY LECTURE	TOPICS

UNIT 2: CYANIDE TOXICITY PROPOSED COURSE OUTLINE

Week	Lecture	Learning outcomes	Practical Exercises
1	Overview of: Cyanide Potential sources and use	At the end of the topic you are expected to know: • What is cyanide • What cyanide is used for • What are the sources of cyanide	Tutorial on overview of cyanide/ quiz
2	Cyanide Toxicity in Human: Acute and chronic poisoning	At the end of the topic you are expected to: • Diagnose cyanide toxicity • Recognize the sign and symptoms of cyanide toxicity • Know what is acute cyanide poisoning • Know what is chronic cyanide poisoning	Pictorial exhibits of classical toxicity and/or Cyanide poisoning
3	Overview of enzymes and proteins	At the end of the topic you are expected to know: • What are enzymes • What are proteins and/or macromolecules • What are the properties of enzymes • What role enzymes play in biological systems	Models depicting enzymes and/or proteins
4	Pathophysiology: Biochemical basis of cyanide toxicity, Highlights of enzymes, proteins, and macromolecules that are affected	At the end of the topic you are expected to know: • What is the biochemical basis of cyanide toxicity • What are the biological targets for cyanide toxicity • What are the tests used to assess cyanide toxicity • What are the biological fluids used to assay possible cyanide toxicity • How do you interpret cyanide results	Pictorial exhibit of enzymes and proteins
5	Clinical features of Cyanide toxicity, Mortality/Morbidity of Cyanide toxicity, Laboratory assessment procedures and findings of possible toxicity	At the end of the topic you are expected to know: • What are the signs and symptoms of cyanide toxicity • What are the typical features and presentations of cyanide poisoning • What is the difference between acute and chronic cyanide toxicity • What are the pathologies associated with cyanide poisoning	1. Diagnosis procedures, recognition of signs and symptoms 2. Exhibits and Case presentations of classical cyanide intoxication Teaching aids to be used are: Pictures, flowcharts, diagrams, illustrations and pathways exhibit

6	Biological detoxification of cyanide: Discussion of Enzymes and Proteins involved	At the end you are expected to know: • What are the enzymes needed to detoxify cyanide toxicity • What are the proteins and macromolecules that play a role in detoxification and their locations	Tutorial on biological defence systems and processes against intoxication
7	Preventative measures against Cyanide poisoning: Safety issues	At the end of the topic you are expected to know: • What are the safety procedures used to avoid contamination • The different safety gears that are available to use when handling cyanide • How prevent contamination in the first place • What are industry best practices regarding toxic substances	Demonstration/exhibit of safety equipments/gears, proper and correct usage, Safety instructions and procedures
8	Treatment Summary: Overview of First Aid (FA), Aims of FA, FA course on cyanide toxicity, Supportive treatment: attendance to and/or application of FA,	At the end of the topic you are expected to know: • Basic general first aid procedures How to apply first aid during a possible cyanide poisoning incident	Demonstration of First Aid Procedures and Treatment at the site of the accidents/intoxication Exhibit of basic First Aid kit and its content that is relevant for emergencies in alluvial miners
9	Overview of Antidote treatment	At the end of the topic you are expected to know: • What are the current antidotes to treat cyanide poisoning • How effective are the various treatment for cyanide poisoning • What are the treatment and antidotes used to manage casualties • Overview of Oxygen, Sodium thiosulfate and Sodium nitrite as antidotes treatment for cyanide poisoning	Tutorial and topic review/quizzes
10	Hospital Treatment and Management: Severe, moderate, and mild poisoning	At the end of the topic you are expected to know: • How to manage a casualty of cyanide poisoning • What the management procedures and regimes are • Recognize and classify whether a particular accident is severe, moderate, or mild case of cyanide poisoning	Demonstration of treatment procedures for different degree of poisoning
11	Analytical Aspects: Summary of methods used to determine cyanide and cyanide intoxication	At the end you are expected to know: • What are the tests and assays used to assess cyanide toxicity • What are reference values • What biological fluids/specimens are used to assess cyanide toxicity	Exhibit/Tutorial to summarize and/or demonstrate the various analytical techniques and procedures
12	Summary overview of current Trends and Developments in Cyanide	At the end you are expected to: • Summarize the efficacy of the various treatments for cyanide	Tutorial/quizzes exercise

	antidotes	poisoning	
13	Field trips to Prospecting sites: Education awareness of cyanide toxicity, Presentation in English and accurate translation in Pidgin	At the end you are expected to: • Appreciate the importance of cyanide as an occupational hazard • Able to translate and interpret basic instructions in pidgin	Field Trip and awareness exercises to educate local prospectors
14	BASIC QUIZZ ON	CYANIDE LECTURE	TOPICS

UNIT 3: HYDROCARBON TOXICITY COURSE OUTLINE

Week	Lecture Topics	Learning Outcomes	Practical Exercises
1	Overview of Hydrocarbons:	At the end of the topic you are expected to know: • What Hydrocarbons are • What is Kerosene • What is Petrol • What is Benzene • What are Detergents • What are Insecticides • What are the potential sources and use of Hydrocarbons	Pictorial of different Hydrocarbons: Petrol, Benzene, Insecticides, Detergents, etc. Show examples/exhibits of common Detergents and Insecticides used in PNG
2	Kerosene/Petrol poisoning in Human: Acute and chronic poisoning	At the end of the topic you are expected to know: • What acute hydrocarbon poisoning • What is chronic hydrocarbon poisoning	Pictorial exhibits of classical toxicity and/or hydrocarbon poisoning
3	Overview of enzymes and proteins	At the end of the topic you are expected to know: • What enzymes are • What proteins and/or macromolecules are	Models depicting enzymes and/or proteins
4	Pathophysiology: Biochemical basis of hydrocarbon toxicity	At the end of the topic you are expected to know: • What is the biochemical basis of hydrocarbon poisoning • What are the enzymes and /or proteins that are affected in hydrocarbon toxicity	Demonstration of attacks to tissues and cells by elevated levels of hydrocarbons, Tutorial on Basic enzymology
5	Clinical presentations and features of kerosene/petrol, and other hydrocarbons toxicity, Mortality/Morbidity of hydrocarbon toxicity, Laboratory assessment procedures and findings of possible toxicity	At the end of the topic you are expected to know: • What are the clinical features of hydrocarbon toxicity • What are the signs and symptom associated hydrocarbon toxicity • What are the target tissues for hydrocarbon toxicity • What the morbidity and mortality rate of hydrocarbon toxicity • How to assess possible toxicity and know laboratory procedures and assessment • Interpret laboratory results in possible Hydrocarbon toxicity	1. Diagnosis techniques, recognition of signs and symptoms 2.Exhibits and Case presentations of classical hydrocarbon intoxication Pictures, flowcharts, diagrams, illustrations and pathways exhibit
6	Biological detoxification of hydrocarbon:	At the end of the topic you are expected to know: • The various biological systems, proteins, enzymes that are play a role in detoxification of hydrocarbons in tissues	Tutorial on biological defence systems and processes against detoxification

7	Preventative measures against hydrocarbon poisoning	At the end of the topic you are expected to know: • Best industry practices • The safety gears and their proper use • Procedures that will minimize occupational hazard	Demonstration/exhibit of safety equipments/gears, proper and correct usage, Safety instructions and procedures
8	Treatment Summary: Overview of First Aid (FA)	At the end of the topic you are expected to know: • First aid procedures and treatment in hydrocarbon poisoning • Importance of adhering to safety procedures and knowing the aims of first aid • Importance of supportive treatment supportive treatment	Demonstration of First Aid Procedures and Treatment at the site of the accidents/intoxication Exhibit of basic First Aid kit and its content that is relevant for emergencies in the alluvial mining sector
9	Antidote treatment: Overview of antidotes	At the end of the topic you are expected to know: • What are the antidotes, if any, used treat hydrocarbon intoxicated • Summary of the efficacy of antidotes, if any, and preventative measures during toxicity	Tutorial and topic review/quizzes
10	Hospital Treatment: Types of poisoning	At the end of the topic you are expected to know: • The different levels of intoxication, i.e., what is moderate, severe, and mild hydrocarbon poisoning	Tutorial on Hospital Treatment of types of poisoning
11	Analytical Aspects: Summary of methods used to determine hydrocarbon intoxication (a) Kerosene (b) Petrol/Gasoline (c) Benzene (d) Insecticides (e) Detergents	At the end of the topic you are expected to know: • How to assess and diagnose kerosene poisoning • How to diagnose petrol poisoning • How to analyse biological fluid for hydrocarbon toxicity • What are the methods used to determine hydrocarbon levels in blood	Exhibit/Tutorial to summarize and/or demonstrate the various techniques
12	Trends and Developments in Hydrocarbon antidotes	At the end of the topic you are expected to know: • Summary of current trends and developments in hydrocarbon antidotes	Tutorial/quizzes exercise
13	BASIC QUIZZ ON	HYDROCARBON	LECTURE TOPICS

Conclusion:

By designing such a program, it is hoped that the Wau Ecological Institute and Department of Mining would have addressed a long standing deficit in knowledge amongst Health workers on substance toxicity, and the local communities that they directly deal with. As such, the intent of such a program, following the necessary reviews and suggestions from various stakeholders, would be for an approval by the NDOH Health Curriculum Advisory Committee for its incorporation into the Community Health Workers curriculum in the country.

Reference:

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