EFFECTS OF DIETARY SUPPLEMENTS OF *Corchorus olitorus* AND *Telfairia occidentalis* ON CYANIDE POISONING IN *Rattus rattus*.

BY

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CERTIFICATION

I certify that this work was carried out by Olayinka Olufunke Olabode in the Department of Epidemiology, Medical Statistics and Environmental Health, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan.

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DEDICATION

This research work is dedicated to the Alpha, the Omega, the Almighty GOD, The One who has been my strength and has made this work a success. He alone deserves all the glory, honour and praise forever.

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ABSTRACT

Many people are exposed to the risk of cyanide poisoning in Nigeria through consumption of cyanide containing foods. *Telfairia occidentalis and Corchorus olitorius* contain cysteine and methionine which have some detoxifying effects on cyanide poisoning. However, there is dearth of information about the ameliorating effects of these vegetables on cyanide poisoning when used as dietary supplements in animal models. The study therefore assesed the efficacy of these two vegetables on cyanide poisoning in *Rattus rattus*.

Thirty male albino Wistar rats of 7weeks old were fed on commercial rat pellets and water *adlibitum* for four weeks. They were randomly allocated to five treatments and one control groups. Lyophilized water extracts of *Telfairia occidentalis and Corchorus olitorius* were reconstituted in water to give a concentration of 3mg/l. The groups were treated with Potassium cyanide (KCN) (3mg/kg) and aqueous vegetable extract (3mg/l) as follows: distilled water only (Control group 1); Diline aqueous KCN only (group 2); KCN and *Telfairia occidentalis* extract (group 3); KCN and *Corchorus olitorius* extracts (group 4); *Telfairia occidentalis* extract only (group 5); *Corchorus olitorius* extracts only (group 6). Physical changes, body weight, ocular lesion and nasal discharge were documented .Biochemical analysis involving detecting levels of Alanine Amino Transaminase (ALT), Aspartate Transominase (AST) and Alkaline Phosphatase (ALP) were used as indicators for liver damage. Sections of the brain, liver and kidney were examined morphologically. The results were analyzed using descriptive statistics and ANOVA.

The mean rat weight change were 24.0±47.6 (Control group), -7.0±19.7 (CN only), 0.0±33.5 (CN + *Telfairia occidentalis* extract), -5.0±10.5 (CN + *Corchorus olitorius* extracts), 3.3±10.3 (*Telfairia occidentalis* extract only) and 12.0± 20.4g (*Corchorus olitorus* extracts only) for rats in groups 1 to 6 respectively ($p<0.05$). In group 3 (CN + *Telfairia occidentalis* extract), 17.1% of the rats had ocular lesion while ocular lesion occurrence was 28.6% in group 4 (CN + *Corchorus olitorus* extracts) and 67.1% in group 2 (CN only) ($p<0.05$). Slimy nasal discharge was found in 22.9% of rats in group 4 (CN + *Corchorus olitorus* extracts) and 28.6 % in group 2 (CN only). No discharge was found in groups 1 (Control group), group 3(CN + *Telfairia occidentalis* extract), group 5 (*Telfairia occidentalis* extract only) and group 6 (*Corchorus olitorus* extracts only). Ranges of values for ALT were 12.69 U/L (units/liter) (Control group), 13.78 U/L (CN only), 15.63 U/L(CN + *Telfairia occidentalis* extract), 22.74 U/L(CN + *Corchorus olitorus* extracts) and 2.69 U/L (*Telfairia occidentalis* extract only) and 7.70

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UL(*Corchorus olitorius* extracts only) for rats in groups 1 to 6 respectively, indicating liver damage in groups 2 (CN only) and group 4 (CN + *Corchorus olitorius* extracts). Histopathological analysis indicated that cyanide caused the following changes in the rats: liver multifocal degeneration, necrosis and slight congestion of the kidney and brain in the rats in group 2 (CN only); mild congestion of the kidney with no visible lesion of the brain and kidney was observed in the rats of group 3 (CN + *Telfairia occidentalis* extract); focal hepatic degeneration and necrosis with no visible lesion in the brain were observed in rats of group 4 (CN + *Corchorus olitorius* extracts). No visible lesion of the liver and brain were observed in rats of group 5 (*Telfairia occidentalis* extract only) and group 6(*Corchorus olitorius* extracts only). All rats in group 1 (control group) had normal values for all assessed parameters.

Telfairia occidentalis and Corchorus olitorius reduced cyanide toxicity in the rats fed with them implying that they have detoxification properties. *Telfairia occidentalis* however had more detoxification effects.

Keywords: Cyanide poisoning, *Rattus rattus*, *Corchorus olitorius*, *Telfairia occidentalis*, Detoxification

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To my mum and dad. I can't thank you enough for your care, love and concern, financial support and endless prayers all these years you are the best parents in the world. I promise you my academic quest will not just stop here it will continue and I will always make you proud. You will live long to see me succeed and enjoy the fruits of your labour. To my siblings Oluwabusola, Oluwasanmi and Anuoluwapo, thanks for your prayers and recharge cards may our strong cord of unity and love never be broken. I also want to extend my sincere appreciation to my mother- in- law for her daily prayers and my brothers and sisters-in- laws for there words of encouragement.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Cyanides are both man-made and naturally occurring substances. In nature they are found in several plant species as cyanogenic glycosides and are produced by certain bacteria, fungi, algae and particularly cassava. It is produced by over 1,000 plant species including sorghum, bamboo and cassava. Relatively low concentrations of cyanide can be highly toxic to people and wildlife (ATSDR, 1989). Cassava accounts for 41.5 per cent of the food consumed in Ogun, Ekiti, Osun, Oyo and Ondo States Former Western Region), as compared to 53 per cent in Ilesdel State (Midwest) and 45 per cent in Anambra and Imo States (East Central) (Okigbo, 1990). Cassava supplies the bulk of the energy intake in Southern Nigeria as compared to other staples; there are several cassava-based food preparations for different periods of the day and various occasions (Okigbo, 1990).

1.2 SOURCES OF CYANIDE

1.2.1 MAN MADE SOURCES AND USES

Cyanide is released into the environment from numerous sources as a result of human activities. Metal finishing and organic chemical finishing industries as well as iron and steel production are major sources of cyanide releases to the aquatic environment. More than 90% of emissions to the air are attributed to releases in automobile exhaust (ATSDR, 1989). Workers in a wide variety of occupations may be exposed to cyanide. The general population may also be exposed to cyanide by inhalation of contaminated air, ingestion of a variety of foods or contaminated drinking water (ATSDR, 1989). Anthropogenic sources of cyanide released into the environment are diverse. Humans are exposed to gas, liquid and solid forms of cyanide from a broad range of natural, industrial and anthropogenic sources. Many chemical forms of cyanide are also used in industrial applications or are present in the environment. The cyanide anion (CN^-) is the primary toxic chemical regardless of origin (WHO, 2004). Sources of cyanide releases from industries include chemical manufacturing and processing plants such as metallurgical and metal plating industries and extraction of gold and

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silver from low-grade ores. Other sources include volatilization from cyanide wastes disposed off in landfills and waste ponds, emissions from municipal solid waste incinerators, biomass burning and fossil fuels combustion including vehicle emissions, fumigation operations and the production of coke or other coal carbonization procedure (WHO, 2004).

Hydrogen cyanide is a product of combustion, including the exhaust of internal combustion engines, tobacco smoke, and especially some plastics derived from acrylonitrile (because of the latter effect, house fires can result in poisonings of the inhabitants.) Potassium ferrocyanide is used to achieve a blue color on cast bronze sculptures during the final finishing stage of the sculpture. On its own, it will produce a very dark shade of blue and is often mixed with other chemicals to achieve the desired tint and hue. It is applied using a torch and paint brush while wearing the standard safety equipment used for any painting application: rubber gloves, safety glasses, and a respirator. The actual amount of cyanide in the mixture varies according to the recipes used by each foundry (ATSDR, 2006).

a) Mining

Gold and silver cyanides are among the very few soluble forms of these metals, and cyanides are thus used in mining as well as electroplating, metallurgy, jewelry, and photography. In *cyanide process* (for the mining of gold and silver), finely ground high-grade ore is mixed with cyanide (concentration of about two kilogram NaCN per tonne); low-grade ores are stacked into heaps and sprayed with cyanide solution (concentration of about one kilogram NaCN per ton). The precious-metal cations are complexed by the cyanide anions to form soluble derivatives, e.g. $[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$.

$$2Au + 4KCN + \tfrac{1}{2}O_2 + H_2O \rightarrow 2K[Au(CN)_2] + 2KOH$$

$$2Ag + 4KCN + \tfrac{1}{2}O_2 + H_2O \rightarrow 2K[Ag(CN)_2] + 2KOH$$

Silver is less "noble" than gold and often occurs as the sulfide, in which case redox is not invoked (no O_2 is required), instead a displacement reaction occurs:

$$Ag_2S + 4KCN \rightarrow 2K[Ag(CN)_2] + K_2S$$

The "pregnant liquor" containing these ions is separated from the solids, which are discarded to a tailing pond or spent heap, the recoverable gold having been removed. The metal is recovered from the "pregnant solution" by reduction with zinc dust or by adsorption onto activated carbon. This process can result in environmental and health problems. Aqueous

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cyanide is hydrolyzed rapidly, especially in sunlight. It can mobilize some heavy metals such as mercury if present. Gold can also be associated with arsenopyrite (FeAsS), which is similar to iron pyrite (fool's gold), wherein half of the sulfur atoms are replaced by arsenic. Au-containing arsenopyrite ores are similarly reactive toward cyanide.

b) Fishing

Cyanides are illegally used to capture live fish near coral reefs for the aquarium and seafood markets (ATSDR. 2006). This fishing occurs mainly in the Philippines, Indonesia and the Caribbean to supply the 2 million marine aquarium owners in the world. In this method, a diver uses a large, needleless syringe to squirt a cyanide solution into areas where the fish are hiding, stunning them so that they can be easily gathered. Many fish caught in this fashion die immediately, or in shipping (ATSDR. 2006). Those that survive to find their way into pet stores often die from shock, or from massive digestive damage. The high concentrations of cyanide on reefs on which this has occurred has resulted in cases of cyanide poisoning among local fishermen and their families, as well as irreversible damage to the coral reefs themselves and other marine life in the area(ATSDR. 2006).

c) Fumigation

Cyanides are used as insecticides for the fumigating of ships. In the past cyanide salts have and still are in some places being used as rat poison (ATSDR. 1993).

d) Execution

Hydrogen cyanide has been used in gas chamber executions (Bokanga et. al., 1994).

1.3 NATURAL SOURCES

Cyanides can be produced by certain bacteria, fungi, and algae, and are found in a number of foods and plants. Cyanide is found, although in small amounts, in apple seeds and almonds (ATSDR, 2006) In plants, cyanides are usually bound to sugar molecules in the form of cyanogenic glycosides and serve the plant as defense against herbivores. Cassava roots aka manioc- the base from which tapioca is made) contains cyanogenic glycosides (Velter, 2000, Jones 1998).

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1.4 TOXICITY

The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure. The LC50 or LD50 (the concentration or dose that is lethal to 50% of the exposed population) for gaseous hydrogen cyanide is 100-300 parts per million. Inhalation of cyanide in this range results in death within 10-60 minutes, with death coming more quickly as the concentration increases. Inhalation of 2,000 parts per million hydrogen cyanide causes death within one minute (ICMI, 2006). The LD50 for ingestion is 50-200 milligrams, or 1-3 milligrams per kilogram ofbody weight, calculated as hydrogen cyanide. For contact with unbraided skin, the LD50 is 100 milligrams (as hydrogen cyanide) per kilogram of body weight (ICMI, 2006).

1.5 MECHANISM OF TOXICITY OF CYANIDE

Cyanide causes a decrease in the utilization of oxygen in tissues producing a state of histotoxic anoxia. Cyanide can also inhibit several other metallo-enzymes containing for the (most part iron), copper or molybdenum e.g. alkaline phosphatase, carbonic anhydrase. Cyanide causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio shifting from aerobic to anaerobic metabolism. Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle (Rosling, 1994). HCN reduces energy availability in all cells but its effects is always most immediate on the respiratory system and the heart. The lethal dose for an adult, depends on the body weight and nutritional status and this is somewhere between 30 and 210mg of HCN. If the HCN exceeds the limit an individual is able to detoxify or tolerate, death may occur while smaller sub- le thal amounts of cyanide cause acute intoxication. Symptoms of acute cyanide intoxication include rapid respiration; drop in blood pressure, rapid pulse, dizziness mental confusion, diarrhea and convulsion (Rosling, 1994). Chronic effects of cyanide intoxication, has been linked to regular long- term consumption in individuals with poor nutrition.

Death due to cyanide poisoning can occur when the cyanide limit exceeds the limit an individual is able to detoxify. The likelihood of cyanide intoxication from consumption of cassava or bamboo shoots is dependent on body weight and it is possible that a child or person of smaller body weight would not be able to detoxify the cyanide resultant from a meal of inadequately prepared cassava or bamboo shoots. The acute lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg body weight. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man

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(Mlingi et. al., 1995). Long-term consumption of cassava with chronic uptake of cyanoglycosides in sub-acutely toxic doses may be involved in the pathogenesis of certain conditions including the disturbance of thyroid function (goitre) and neuropathies. this thyrotoxic effects of cyanide depends on its conversion to the iodine antagonist thiocyanate (Mlingi et. al., 1995). Human cassava eating population showed ophthalmological and neurological symptoms, which are associated with exposure to HCN.

Other nutritional and metabolic deficiencies affecting the cyanide detoxification mechanism include sulphate and zinc deficiencies. Several epidemiological studies, in cassava eating population had established an association between cyanide exposure and spasticparaparesis, amblyobia ataxia or tropical ataxia neuropathy (TAN) (ATSDR, 2006). Neurological disorders and thyroid abnormalities have been linked with long-term consumption of cassava (Baskin et. al., 1998). Surveys in African communities where cassava is a staple crop show a strong correlation between cassava consumption and endemic goitre and cretinism. Dietary deficiencies, especially low intake of iodine, may contribute to this effect (Oke, 1980). In Nigeria and some other tropical countries in Africa, where the daily diet is dominated by starchy staple foods, dietary cyanide exposure from cyanogenic glycosides in insufficiently processed foods containing HCN glycosides has been implicated as contributing factor in growth retardation. The nutritional interest in some of these vegetable species stems from their rich contents of essential amino acids, vitamins and minerals. Further to their rich content of the mentioned nutrients, it is established that green vegetable leaves are the cheapest and most abundant source of proteins because of their ability to synthesize amino acids from a wide range of virtually available primary materials such as water, carbon dioxide, and atmospheric nitrogen (as in legumes) (Fasuyi, 2006). Therefore, some of these vegetables are the cheapest and most readily available sources of important proteins, vitamins and essential amino acids.

In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate (SCN). This detoxification requires sulphur donors that are provided by sulphur- containing dietary amino acids, cysteine and methionine (Okigbo, 1999). In subjects who have an adequate protein component of their diet, excess cysteine and methionine are not required for protein synthesis and are degraded to inorganic sulphate and excreted.

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1.6 JUSTIFICATION FOR THE STUDY

Many people are exposed to the risk of cyanide poisoning in Nigeria through consumption of cyanide containing food and also exposure to various anthropogenic sources of cyanide poisoning. Though several studies have been carried out on cyanide poisoning and chemotherapy interventions, there is dearth of information about the ameliorating effects of these vegetables (*Telfairia occidentalis* and *Curcuma olitoria*) on cyanide poisoning.

1.7 OBJECTIVE OF THE STUDY

The objective of this study is to determine the effectiveness of *Curcuma olitoria* (Ewedu) and *Telfairia occidentalis* (Ugwu) in the detoxification of cyanide in populations exposed to cyanide intoxication in various forms including consumption of cyanoglycosides using rats as a model.

1.8 SPECIFIC OBJECTIVES

i. To reproduce acute toxic effect(s) of cyanide in-vivo.
ii. To test the efficacy of *Curcuma olitoria* (Ewedu) and *Telfairia occidentalis* (Ugwu) in counteracting the toxic effect of cyanide intoxication in the animal models.
iii. To extrapolate the result from two (2) above to recommend the possibility of using the plants in alleviating sub-acute cyanide poisoning.

1.9 LIMITATION OF THE STUDY

i. The inability of obtaining the sulphur containing amino acids (methionine and cysteine) as standard references.
ii. The inability to estimate the values of the amino acids in the vegetables.
iii. The inability to monitor plasma levels of cyanide in experimental animals.
iv. High cost of experimentation, thus limiting the scope of investigation.

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CHAPTER TWO

LITERATURE REVIEW

2.0 BRIEF DESCRIPTION OF CYANIDE

Cyanides comprise a wide range of compounds of varying degrees of chemical complexity, all of which contain a CN moiety, to which humans are exposed in gas, liquid, and solid form from a broad range of natural and anthropogenic sources. While many chemical forms of cyanide are used in industrial application or are present in the environment, the cyanide anion CN^- is the primary toxic agent, regardless of origin (WHO, 2004).

Hydrogen cyanide is a colourless or pale blue liquid or gas with a faint bitter almond-like odour. It is used primarily in the production of substances such as adiponitrile, methyl methacrylate, chelating agents, cyanuric chloride, methionine and its hydroxylated analogues, and sodium and potassium cyanide. Hydrogen cyanide is also used as a fumigant in ships, railroad cars, large buildings, grain silos, and flour mills, as well as in the fumigation of pests and seeds in vacuum chambers. Other cyanides, such as sodium and potassium cyanide, are solid or crystalline hygroscopic salts widely used in ore extracting processes for the recovery of gold and silver, electroplating, case-hardening of steel, base metal flotation, metal degreasing, dyeing, printing, and photography (WHO, 2004). They are also widely used in the synthesis of organic and inorganic chemicals (e.g., nitriles, carboxylic acids, amides, esters, and amines; heavy metal cyanides) and in the production of chelating agents. Hydrogen cyanide is formed during the incomplete combustion of nitrogen-containing polymers, such as certain plastics, polyurethanes, and wool. Hydrogen cyanide is present in cigarette smoke (WHO, 2004).

2.1 PROPERTIES OF CYANIDE

2.1.1 Physical Properties

Cyanide is considered, in a broad sense, to be the most potent ligand for many transition metals. The very high affinities of metals for cyanide can be attributed to its negative charge, compactness and ability to engage in π-bonding. Well known complexes include:

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- the hexacyanides $[M(CN)_6]^{3-}$ (M = Ti, V, Cr, Mn, Fe, Co), which are octahedral in geometry;
- the tetra cyanides, $[M(CN)_4]^{2-}$ (M = Ni, Pd, Pt), which are square planar in geometry;
- the dicyanides $[M(CN)_2]^-$ (M = Cu, Ag, Au), , which are linear in geometry.

Due to its high nucleophilicity, cyanide is readily introduced into organic molecules by displacement of the corresponding organic halide. Organic c yanides are generally called nitriles. Thus CH_3CN can be methyl cyanide but more commonly is referred to as acetonitrile. In organic synthesis, cyanide is used as a C-1 synthon. I.e., it can be used to lengthen a carbon chain by one, while retaining the ability to be functionalized.

$RX + CN^- \rightarrow RCN + X^-$ (Nucleophilic Substitution) followed by:

1. $RCN + 2\,H_2O \rightarrow RCOOH + NH_3$ (Hydrolysis), or

2. $RCN + 0.5\,LiAlH_4$ + (second step) $2\,H_2O \rightarrow RCH_2NH_2 + 0.5\,LiAl(OH)_4$ (under reflux in dry ether, followed by addition of H_2O)

An alternative method for introducing cyanide is via the process of hydrocyanation, whereby hydrogen cyanide and alkenes combine:

$RCH{=}CH_2 + HCN \rightarrow RCH(CN)CH_3$

Metal catalysts are required for such reactions.

Hydrogen cyanide is a colourless or pale blue liquid with characteristic odour of bitter almond (Verschueren, 1983). It has a molecular weight of 27.03 and a boiling point of 25.6°C (Amoore and Hautala 1983). It is miscible with water and alcohol and slightly soluble in ether (Budavari, 1989). Most people can smell hydrogen cyanide. Due to an apparent genetic trait, some individuals cannot detect the odor of HCN (Bokanga et al., 1991). Sodium cyanide and potassium cyanide are both white powders with a bitter almond-like odor in damp air, due to the presence of hydrogen cyanide formed by hydrolysis:

$NaCN + H_2O \rightarrow HCN + NaOH$

2.1.2 Chemical Properties

Once released in the environment, the reactivity of cyanide provides numerous pathways for its degradation and attenuation:

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a) Complexation

Cyanide forms ionic complexes of varying stability with many metals. Most cyanide complexes are much less toxic than cyanide, but weak acid dissociable complexes such as those of copper and zinc are relatively unstable and will release cyanide back to the environment. Iron cyanide complexes are of particular importance due to the abundance of iron typically available in soils and the extreme stability of this complex under most environmental conditions. However, iron cyanides are subject to photochemical decomposition and will release cyanide if exposed to ultraviolet light.

Metal cyanide complexes are also subject to other reactions that reduce cyanide concentrations in the environment, as described below.

b) Precipitation

Iron cyanide forms precipitates with iron, copper, magnesium, cadmium and zinc over a pH range of 2-11 (ICMI, 2006).

c) Adsorption

Cyanide and cyanide-metal complexes are adsorbed on organic and inorganic constituents in soil, including oxides of aluminium, iron and manganese, certain types of clays, feldspars and organic carbon. Although the strength of cyanide retention on inorganic materials is unclear, cyanide is strongly bound to organic matter (ICMI, 2006).

d) Oxidation

Oxidation of cyanide to less toxic cyanate normally requires a strong oxidizing agent such as ozone, hydrogen peroxide or hypochlorite. However, adsorption of cyanide on both organic and inorganic materials in the soil appears to promote its oxidation under natural conditions (ICMI, 2006).

e) Sulpharation

Cyanide reacts with some sulfur species to form less toxic thiocyanate. Potential sulfur sources include free sulfur and sulfide minerals such as chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and pyrrhotite (Fe S), as well as their oxidation products, such as polysulfides and thiosulfate(ICMI, 2006).

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f) Volatilization

At the pH typical of environmental systems, free cyanide will be predominately in the form of hydrogen cyanide, with gaseous hydrogen cyanide evolving slowly over time. The amount of cyanide lost through this pathway increases with decreasing pH, increased aeration of solution and with increasing temperature. Cyanide is also lost through volatilization from soil surfaces (ICMI, 2006).

g) Biodegradation

Under aerobic conditions, microbial activity can degrade cyanide to ammonia, which then oxidizes to nitrate. This process has been shown effective with cyanide concentrations of up to 200 parts per million. Although biological degradation also occurs under anaerobic conditions, cyanide concentrations greater than 2 parts per million are toxic to these microorganisms (ICMI, 2006).

h) Hydrolysis

Hydrogen cyanide can be hydrolyzed to formic acid or ammonium formate. Although this reaction is not rapid, it may be of significance in ground water where anaerobic conditions exist (ICMI, 2006).

i) Effects of Cyanide on Wildlife

Although cyanide reacts readily in the environment and degrades or forms complexes and salts of varying stabilities, it is toxic to many living organisms at very low concentrations (ICMI, 2006).

j) Aquatic Organisms

Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. Concentrations of free cyanide in the aquatic environment ranging from 5.0 to 7.2 micrograms per liter reduce swimming performance and inhibit reproduction in many species of fish. Other adverse effects include delayed motility, pathology, and susceptibility to predation, disrupted respiration, osmoregulatory disturbances and altered growth patterns. Concentrations of 20 to 76 micrograms per liter free cyanide cause the death of many species, and concentrations in excess of 200 micrograms per liter are rapidly toxic to most species of fish. Invertebrates experience adverse nonlethal effects at 18 to 43 micrograms per liter free cyanide, and lethal

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effects at 30 to 100 micrograms per liter (although concentrations in the range of 3 to 7 micrograms per liter caused death in the amphipod (Gammarus pulex) (ICMI. 2006)

Algae and macrophytes can tolerate much higher environmental concentrations of free cyanide than fish and invertebrates, and do not exhibit adverse effects at 160 micrograms per liter or more. Aquatic plants are unaffected by cyanide at concentrations that are lethal to most species of freshwater and marine fish and invertebrates. However, differing sensitivities to cyanide can result in changes to plant community structure, with cyanide exposures leaving a plant community dominated by less sensitive species (ICMI, 2006)

The toxicity of cyanide to aquatic life is probably caused by hydrogen cyanide that has ionized, dissociated or photo chemically decomposed from compounds containing cyanide. Toxic effects of the cyanide ion itself on aquatic organisms are not believed to be significant, nor are the effects of photolysis of ferro- and ferricyanides. It is therefore the hydrogen cyanide concentration of water that is of greatest significance in determining toxicity to aquatic life rather than the total cyanide concentration. The sensitivity of aquatic organisms to cyanide is highly species specific, and is also affected by water pH, temperature and oxygen content, as well as the life stage and condition of the organism.

k) Birds

Reported oral Lethal Dose 50/1 LD_{50} for birds range from 0.8 milligrams per kilogram of body weight (American racing pigeon) to 11.1 milligrams per kilogram of body weight (domestic chickens). Symptoms including panting, eye blinking, salivation and lethargy appear within one-half to five minutes after ingestion in more sensitive species, and up to ten minutes after ingestion by more resistant species. Exposures to high doses resulted in deep, labored breathing followed by gasping and shallow intermittent breathing in all species. Mortality typically occurred in 15 to 30 minutes; however birds that survived for one hour frequently recovered, possibly due to the rapid metabolism of cyanide to thiocyanate and its subsequent excretion.Sub lethal effects of cyanide exposure to birds, such as an increase in their susceptibility to predators, have not been fully investigated and reported (ICMI. 2006)

l) Mammals

Cyanide toxicity to mammals is relatively common due to the large number of cyanogenic forage plants such as sorghum, Sudan grasses, corn and cassava. Concentrations of cyanide in

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these plants are typically highest in the spring during blooming. Dry growing conditions enhance the accumulation of cyanogenic glycosides in certain plants as well as increase the use of these plants as forage (ICMI, 2006).

2.2 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

2.2.1 Natural Occurrence

Hydrogen cyanide is ubiquitous in nature. It is found in the stratosphere and non-urban troposphere (USEPA, 1990). It is released into the atmosphere from biomass burning, volcanoes, and natural biogenic processes from higher plants, bacteria, algae, and fungi (Fiksel et al., 1981; Cicerone & Zellner, 1983; Way et al., 1984; ATSDR, 1997; Li et al., 2000). An estimate of the amount of cyanide released to the environment from natural biogenic processes is not available (ATSDR, 1997). Cyanide occurs naturally as cyanogenic glycosides in at least 2000 plants see (JECFA, 1993). Known cyanogenic glycosides in plants include amygdalin, linamarin, dhurrin, prunasin, lotaustralin and taxiphyllin. Amygdalin (d-mandelonitrile*beta*-d-glucoside-6-*beta*-d-glucoside) has been found in about 1000 species of plants, including cassava (tapioca, manioc), sweet potato, corn, cabbage, linseed, millet, and bamboo, in pits of stone fruits, such as cherries, peaches, and apricots, and in apple seeds (JECFA, 1993; Sharma, 1993; Padmaja, 1995). It is also present in bitter almonds and American White lima beans (Emmons et al., 1972). Among them, cassava (tapioca, manioc) and sorghum are staple foods for hundreds of millions of people in many tropical countries. After ingestion, linamarin can be hydrolysed by either cassava linamarase or an endogenous *beta*-glucosidase to yield d-glucose (Frakes et al., 1986a). Hydrogen cyanide is released into the atmosphere from natural biogenic processes from higher plants, bacteria, and fungi. In air, cyanide is present as gaseous hydrogen cyanide, with a small amount present in fine dust particles (WHO, 2004).

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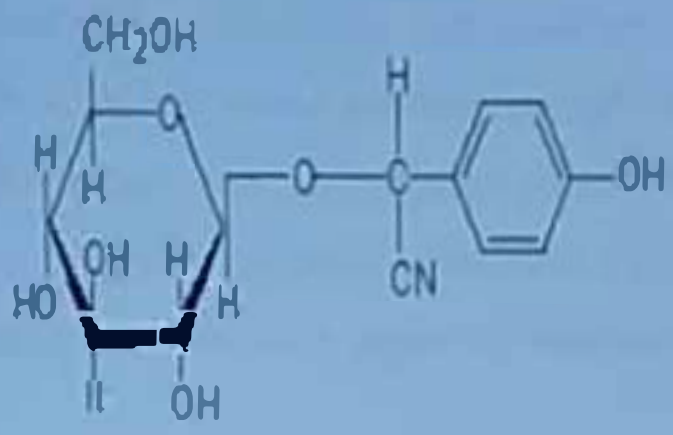
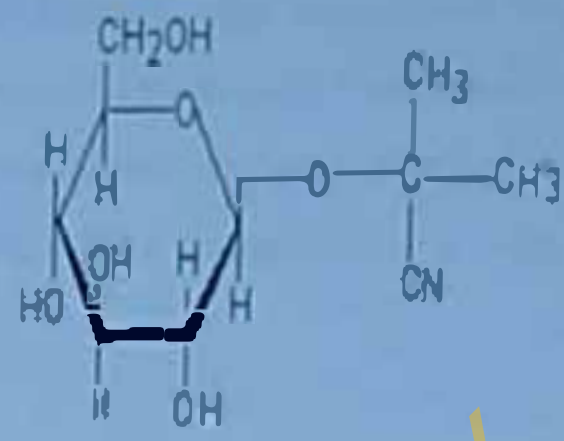
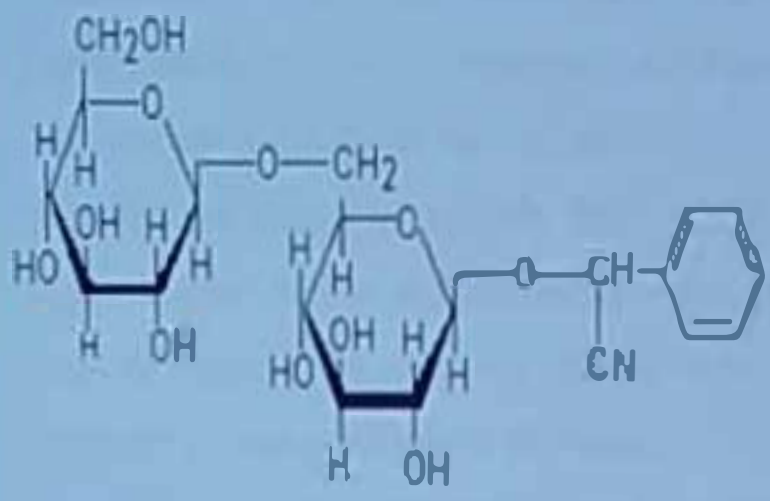
Dhurrin (CAS No. 499-20-7)



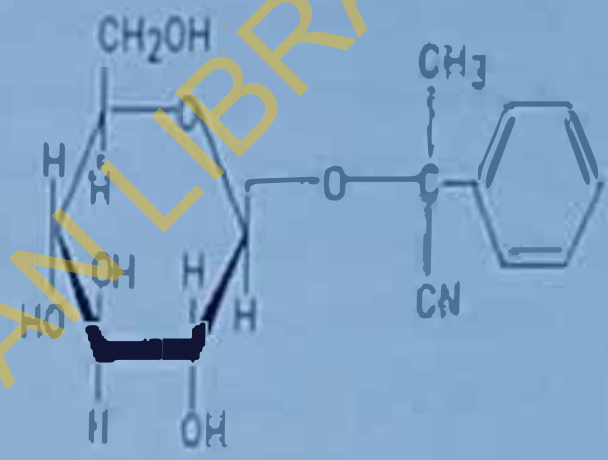
Linamarin (CAS No. 554-35-8)



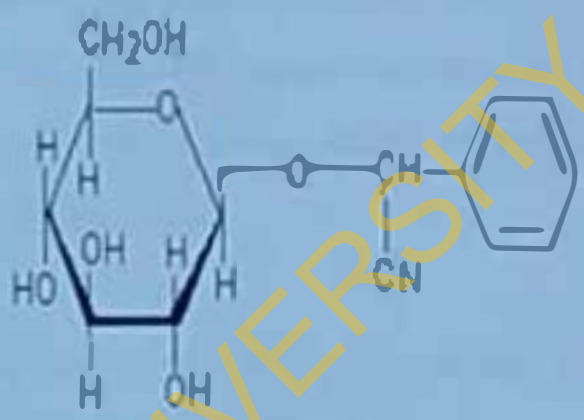
Amygdalin (CAS No. 29883-15-6)



Lotaustralin (CAS No. 534-67-8)



Prunasin (CAS No. 99-18-3)



Taxiphyllin (CAS No. 21401-21-8)

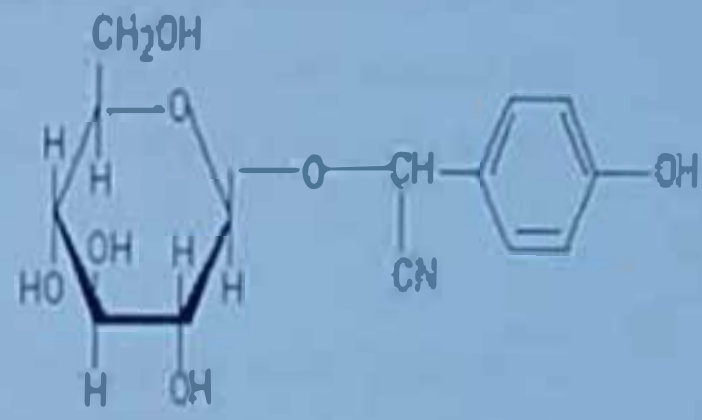


Fig. 2.1: Structures of cyanogenic glycosides in major edible plants (IECFA, 1993)

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2.3 SOURCES OF EXPOSURE TO CYANIDE INTOXICATION

Non-point sources of cyanide released to water can result from runoff from cyanide-containing anti-caking salts used on roads, migration from landfills, and agricultural and atmospheric fallout and washout (ATSDR, 1997). Point sources of releases to water include discharges from gold mining plants, wastewater treatment works, iron and steel production, and organic chemical industries. Cyanides have the potential to be transported over long distances from their respective emission sources (WHO, 2004).

The majority of human population is exposed to very low levels of cyanide in the general environment. There are, however, specific subgroups with higher potential for exposure. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogenic glycosides, such as cassava, specialty foods such as apricot pits, and bitter almonds. Other subgroups with greatest potential for exposure include those in the vicinity of accidental or intended releases from point sources, active and passive smokers, and fire-related smoke inhalation victims. Workers may be exposed to cyanides during fumigation operations and the production and use of cyanides in many industrial processes — for example, electroplating, case-hardening of steel, and extraction of gold and silver from ores (WHO, 2004). One cigarette without a filter liberates 500-µg hydrogen cyanide, while filter cigarettes liberate only 100 µg in mainstream smoke. Hydrogen cyanide concentrations in mainstream and sidestream smoke ranging from 280 to 550 µg/cigarette and from 53 to 111 µg/cigarette, respectively, have been reported; side stream: mainstream ratios of hydrogen cyanide concentrations ranged from 0.06 to 0.50 (ATSDR, 1997).

The level of hydrogen cyanide found in Canadian cigarette smoke under International Organization for Standardization standard smoking conditions were as follows: mainstream smoke, 32–156 µg/cigarette; and side stream smoke, 77–136 µg/cigarette (Health Canada, 2002). The average rate of emission of hydrogen cyanide by automobile exhaust was reported to be 7–9 mg/km for cars not equipped with catalytic converters and on the order of 0.6 mg/km for cars with catalytic converters operating under optimum conditions in the mid- to late 1970s (ATSDR, 1997). Cyanogen chloride is formed as a reaction product of organic precursors with hypochlorous acid in the presence of ammonia and may be formed as a by-product of the chloramination of water (e.g., via the reaction of humic substances with chlorine and chloramine used for water disinfection) (Ohya & Kanno 1987, IPCS, 2000). In

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the USA, 35% of the surface water plants and 23% of the groundwater plants using chloramine as a primary or secondary disinfectant report cyanogen chloride formation (US EPA, 2002). Cyanogen is generated in the combustion of nitrogen-carbon compounds and appears in automobile exhaust gases and gases from blast furnaces (CHEMINFO, 1998).

2.3.1 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Air

Cyanide is found in ambient air as hydrogen cyanide and to a smaller extent in particulate matter. The concentration of hydrogen cyanide measured since 1981 in the northern hemisphere's non-urban troposphere ranged from 180 to 190 ng/m^3 (Cicerone & Zellner, 1983; Jaramillo et al., 1989). Ambient air monitoring data for cyanides in Bulgaria in areas near petrochemical plants showed concentrations ranging from 0.2 to 0.8 µg/m^3 (annual average value) (Kaloyanova et al., 1985). Cyanide has been detected at levels of 20–46 mg/m^3 in the air near large-scale cassava processing facilities in Nigeria (Okafor & Maduagwu, 2000).

Water

Cyanides, reported as cyanide, hydrogen cyanide, sodium cyanide, potassium cyanide, calcium cyanide, or copper (I) cyanide, have been detected in surface water samples at 70 of the 154 hazardous waste sites where they were studied in the USA; they have also been detected in groundwater samples at 191 of the 419 waste sites studied and in leachate samples of 16 of the 52 sites studied (WHO, 2004). The median concentrations in the positive samples were 160 µg/litre for groundwater, 70 µg/liter for surface water, and 479 µg/liter for the leachates (HazDat, 2003). Data from the US National Urban Runoff Program in 1982 revealed that 16% of urban runoff samples collected from four cities across the USA contained cyanides at levels of 2–33 µg/litre (ATSDR, 1997). According to the US Environmental Protection Agency's (EPA) STORET database, the mean cyanide concentration in most surface waters in the USA is less than 3.5 µg/litre. Data from the late 1970s to early 1980s indicated that the levels are higher only in limited areas and may exceed 200 µg/litre (ATSDR, 1997). In 1978, a US EPA survey of drinking-water supplies showed that about 7% of the supplies had cyanide concentrations greater than 10 µg/litre (US EPA, 1993a). Cyanogen chloride is one of the 18 compounds that occur most frequently (8 of 10 city surveys) in potable water within the framework of the US National Organic Reconnaissance Survey (Reding et al., 1982). In a survey in 1987 of over 35 drinking-water

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supplies, the quarterly median cyanogen chloride concentrations in drinking water ranged from 0.15 to 0.80 µg/litre (from 0.19 to 0.34 µg cyanide/litre) (Krasner et al., 1989; ATSDR, 1997). More current data regarding the cyanide and cyanogen chloride levels in drinking water are lacking. Levels of 1.58–7.89 mg cyanide/litre have been found in natural water sources near large-scale cassava processing facilities in Nigeria (Okafor et al., 2001).

Soil

Cyanide has been identified in the soil of hazardous waste sites in the USA; the median concentrations for the positive sites were 0.8 mg/kg in the subsurface soil (found at 77 sites of the 124 studied) and 0.4 mg/kg in the topsoil (51 positive sites out of 91 sites) (HazDat, 2003). Cyanide-containing wastes are commonly found in soils at former manufactured gas plant sites in the USA. Most concentrations of cyanide compounds at the manufactured gas plant sites are below 2000 mg/kg. The most prevalent types of cyanide compounds are iron-complexed forms, e.g., ferric ferrocyanide (Prussian blue), rather than the highly toxic free cyanide forms. Iron-complexed cyanides, dominated by the ferrocyanide ion, comprise over 97% of total cyanides in either weathered or un-weathered soils (Shifrin et al., 1996).

Food

Many edible plants contain cyanogenic glycosides, whose concentrations can vary widely as a result of genetic and environmental factors, location, season, and soil types (Ermans et al., 1980; JECFA, 1993). Some of the foodstuffs and their cyanide contents are shown in Table 1. Cassava tubers vary widely in their cyanogenic glycoside content, although most varieties contain 15–100 mg cyanide/kg fresh weight. Occasionally varieties of cassava tubers contain 1300–2000 mg cyanide/kg fresh weight, and cassava leaves contain 1000–2000 mg cyanogenic glucosides/kg, on a dry matter basis (Padmaja, 1995). Fermentation of cassava pulp for 96 h during garri production reduced the hydrogen cyanide content by 50%; soaking of sliced cassava for 24 h, 40%; and sun drying, some 15% (Kendirim et al., 1995). It should be noted that the ranges of cyanide concentrations shown in Table 1 are very broad in several cases (i.e., cereals and their products, soy protein products, and apricot pits), which may be due to their different sources and differences in analytical procedures; as well, the values may reflect the older literature (WHO, 2004).

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Table 2.1: Cyanide Concentrations in Food Products.

Type of product	Cyanide concentration (in mg/kg or mg/liter)
Cereal grains and their products	0.001-0.45
Soy protein products	0.07-0.3
Soybean hulls	1.24
Apricot pits, wet weight	89-2170
Home-made cherry juice from pitted fruits	5.1
Homemade cherry juice containing 100% crushed pits	23
Commercial fruit Juices	
Cherry	4.6
Apricot	2.2
Prune	1.9
Tropical foodstuffs	
Cassava (bitter) / dried root cortex	2360
Cassava (bitter) / leaves	300
Cassava (bitter) / whole tubers	380
Cassava (sweet) / leaves	451
Cassava (sweet) / whole tubers	445
Gari flour (Nigeria)	10.6-22.1
Sorghum / whole immature plant	2400
Bamboo / immature shoot tip	7700
Lima beans from Java (colored)	3000
Limabeans from Puerto Rico (black)	2900
Lima beans from Burma (white)	2000

From Narley, (1980); Honig et al., (1983); JECFA, (1993); ATSDR, (1997).

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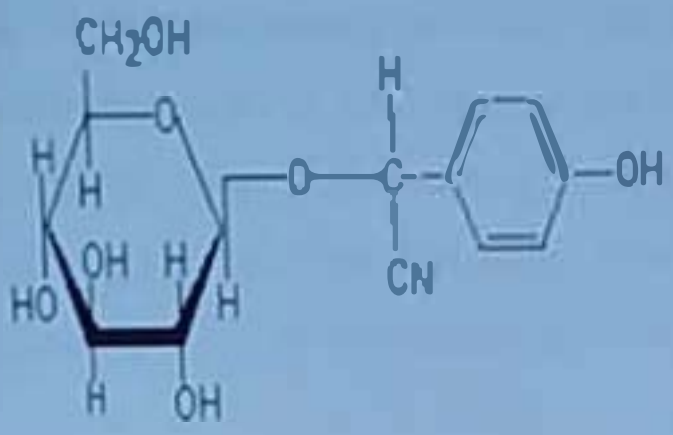
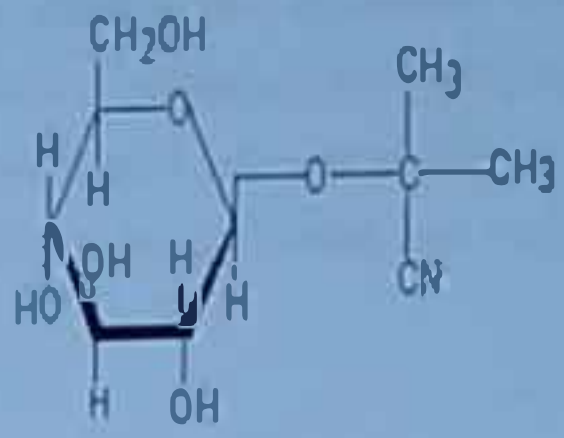
Dhurrin (CAS No. 499-20-7)



Linamarin (CAS No. 554-35-8)



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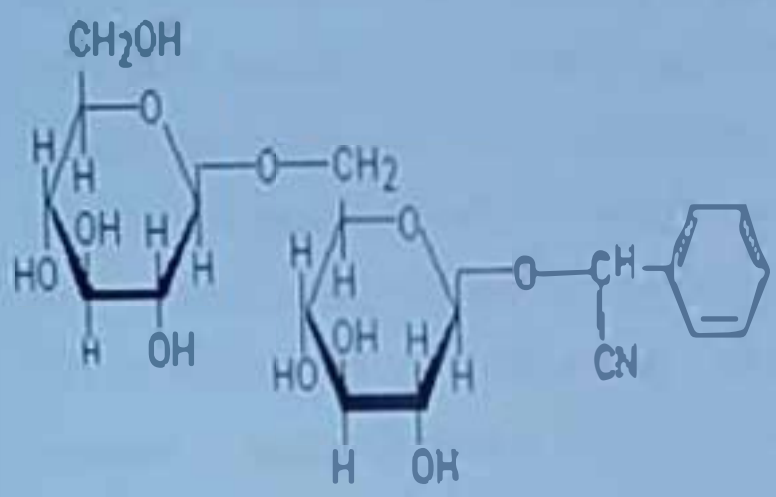

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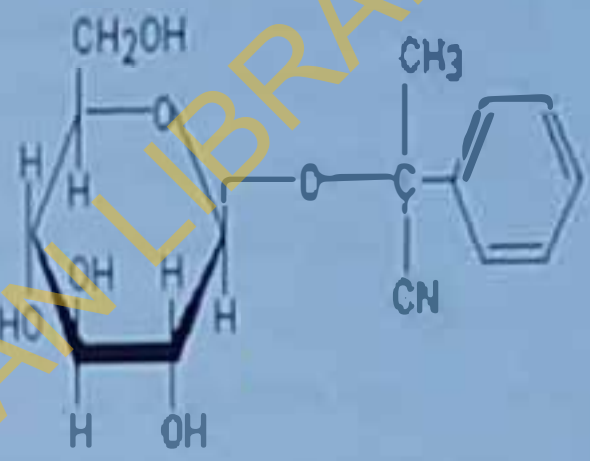

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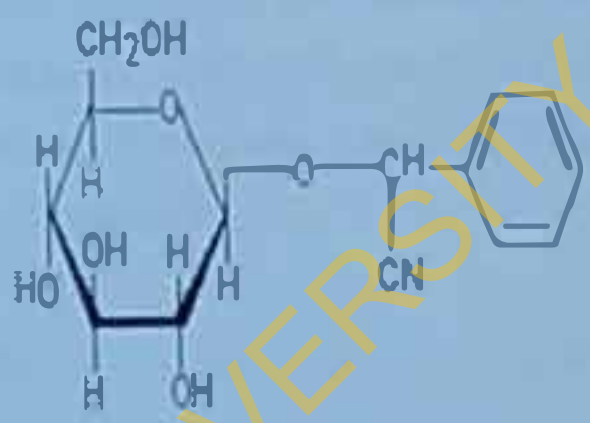

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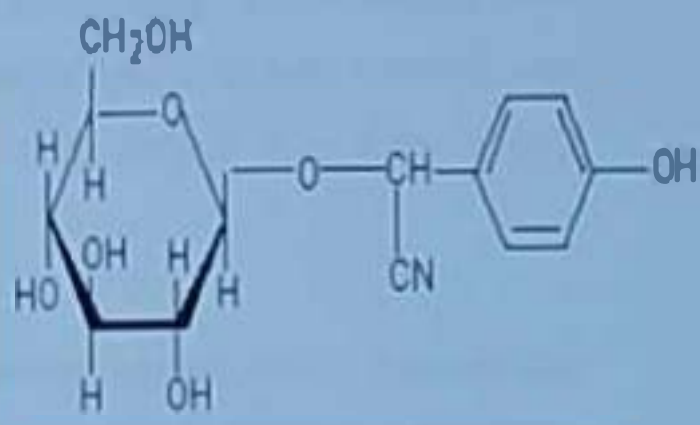

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Gari flour (Nigeria)	10.6-22.1
Sorghum / whole immature plant	2400
Bamboo / immature shoot tip	7700
Lima beans from Java (colored)	3000
Lima beans from Puerto Rico (black)	2900
Lima beans from Burma (white)	2000

From Nartey, (1980); Honig et al., (1983); JECFA, (1993); ATSDR, (1997).

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Human exposure to cyanide by dietary intake is estimated to be potentially of major significance for cassava-consuming populations; cassava has been estimated to be the staple food for 500 million people (WHO, 2004). However, data on the concentrations of cyanides in the total diet are lacking; hence, the daily cyanide intake from food cannot be calculated. For human consumption, cassava can be eaten raw, cooked, or grated and roasted into flour and eaten as "garri," which is the common form in Nigeria (Kendirim et al., 1995). In Mozambique, it was estimated that in families affected by the "mantakassa" disease (spastic paraparesis), the daily intake of cyanogens was 14–30 mg (as cyanide) at the time of a mantakassa epidemic in 1981 (Ministry of Health, Mozambique, 1984). In Nigeria, it was estimated that the intake of hydrogen cyanide in the tropical ataxia-endemic areas may be as high as 50 mg/day (Osuntokun, 1981).

Hydrogen cyanide can be produced by hydrolytic reaction catalysed by one or more enzymes from the plants containing cyanogenic glycosides. In kernels, for example, this reaction is catalysed by the enzyme emulsin (Lasch & El Shawa, 1981) when the seeds are crushed and moistened. Amygdalin (which is also present in cassava, bitter almonds, and peach stones) is converted to glucose, benzaldehyde, and hydrogen cyanide (Figure 2.1) (IPCS, 1992).

Hydrogen cyanide release can occur during maceration of foods containing cyanide, which activates intracellular *beta*-glucosidases. This reaction can also result from chewing, which causes the enzyme and the cyanogenic glycosides stored in different compartments to combine (Ermans et al., 1980; Nahrstedt, 1993). The reaction occurs rapidly in an alkaline environment, and the hydrolysis is complete in 10 min. Hydrolysis is possible in an acid solution and takes place slowly. Liberation of hydrogen cyanide from cyanogenic glycosides occurs usually after ingestion and hydrolysis by the glycosidases of the intestinal microflora and, to a lesser degree, by glucosidases of the liver and other tissues (Padmaja, 1996). However, hydrolysis may also occur during the preparation of the food, which may account for the short interval between ingestion and the appearance of signs of poisoning in some accidents (Lasch & El Shawa, 1981).

It has also been shown in humans that a substantial part of the ingested linamarin is absorbed and excreted intact in the urine (Brimer and Rosling 1993). Its toxic role remains speculative but one is certain that the cyanide liberated from linamarin is the primary cause of toxicity in

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Figure 2.2: Hydrolysis of amygdalin

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cassava. When linamarin comes into contact with its hydrolytic enzyme, linamarase, the molecule is split into glucose and its aglycone, acetone cyanohydrin. The latter can be further degraded by another enzyme or spontaneously under alkaline conditions to form hydrogen cyanide and glucose (Fig 2.2). Thus if the residual linamarin and its breakdown products are not removed during food processing, they may be retained in the foodstuff. It is believed that in humans, linamarin can be broken down by linamarase found in the bacteria that reside in the intestinal track resulting in release of hydrogen cyanide. Fortunately, humans can readily neutralize about 10 mg of cyanide by a reversible reaction with methemoglobin fraction in the red blood cells (Lundquist et al 1985). Rhodanese can further convert majority of the cyanide to less toxic thiocyanate, which is then excreted in the urine.

The principal features of the toxicity profile for cyanide are its high acute toxicity by all routes of administration, with a very steep and rate-dependent dose-effect curve, and chronic toxicity, probably mediated through the main metabolite and detoxification product, thiocyanate. The toxic effects of cyanide ion in humans and animals are generally similar and are believed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration and consequent histotoxic anoxia. The primary targets of cyanide toxicity in humans and animals are the cardiovascular, respiratory, and central nervous systems. The endocrine system is also a potential target for long-term toxicity, as a function of continued exposure to thiocyanate, which prevents the uptake of iodine in the thyroid and acts as a goitrogenic agent.

In humans, whereas slight effects occur at exposure levels of 20–40 mg/m^3, 50–60 mg/m^3 can be tolerated without immediate or late effects for 20 min to 1 h. 120–150 mg/m^3 may lead to death after 0.5–1 h. 150 mg/m^3 is likely to be fatal within 30 min, 200 mg/m^3 is likely fatal after 10 min, and 300 mg/m^3 is immediately fatal. The lowest reported oral lethal dose for humans is 0.5-1-mg/kg-body weight, and the average absorbed dose at the time of death has been estimated at 1.1-mg/kg body weight (calculated as hydrogen cyanide). Sequelae after severe acute intoxications may include neuropsychiatric manifestations and Parkinson-type disease. Cyanide from tobacco smoke has been implicated as a contributing factor in tobacco alcohol amblyopia. Long-term exposure to lower concentrations of cyanide in occupational settings can result in a variety of symptoms related to central nervous system effects.
Long-term consumption of cassava containing high levels of cyanogenic glycosides has been associated with tropical ataxic neuropathy, spastic paraparesis, and, in areas with low iodine

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intake, development of hypothyroidism, goitre, and cretinism (WHO, 2004). While exposure to cyanide has been crudely estimated to be 15–50 mg/day in endemic areas in some such cases, owing to the limitations of data on exposure and potential impact of confounders such as malnutrition, low protein content of the diet, vitamin deficiencies, and iodine status, the available data do not provide meaningful information on dose–response for cyanide (WHO, 2004). Data on end-points other than acute toxicity are somewhat limited. This is attributable in large part to difficulties in conducting, for example, investigations of repeated-dose or chronic toxicity due to the high acute toxicity of the compound. Cyanides are weakly irritating to the skin and eye; data on sensitizing properties or carcinogenicity of hydrogen cyanide or its alkali salts have not been identified. Although somewhat limited, the weight of evidence of available data indicates that cyanide is not genotoxic and that it induces developmental effects only at doses or concentrations that are overtly toxic to the mothers (WHO, 2004). Available data in human populations are considered inadequate as a basis for characterization of dose–response for chronic ingestion of cyanide.

In a 13-week repeated-dose toxicity study in which cyanide was administered in drinking-water, there were no clinical signs associated with central nervous system effects or histopathological effects in the brain or thyroid of rats or mice exposed to doses up to 12.5 mg and 26 mg cyanide/kg body weight per day, respectively. At 12.5 mg cyanide/kg body weight per day, there were slight changes in the reproductive tract in male rats which, although they apparently would not affect fertility in rats, are possibly significant to humans. The no-observed-adverse-effect level (NOAEL) for these effects was 4.5-mg/kg body weight per day (WHO, 2004). The examination of neurotoxicity in this study was limited to clinical observation and optical microscopy in autopsy. The few available studies specifically intended to investigate neurotoxicity, while reporting adverse effects at exposure levels of 1.2 mg cyanide/kg body weight per day in rats and 0.48 mg cyanide/kg body weight per day in goats, suffer from weaknesses that preclude their quantitative assessment (WHO, 2004).

In relation to characterization of concentration–response for repeated-dose toxicity for inhalation (relevant principally to the occupational environment), in three separate studies in rats, there were no adverse systemic effects in rats exposed to acetone cyanohydrin, which is rapidly hydrolysed to hydrogen cyanide at physiological pH, at concentrations up to 211 mg/m^3 (corresponding to a concentration of 67 mg hydrogen cyanide/m^3) (WHO, 2004). The steepness of the dose–effect curve is illustrated by the observation of 30% mortality among

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rats exposed part of the day to 225 mg acetone cyanohydrin/m^3 (71 mg hydrogen cyanide/m^3). Adverse effects of exposure to the low concentrations of cyanide that are generally present in the general environment (<1 $\mu g/m^3$ in ambient air; <10 µg/litre in water) are unlikely. Acute cyanide intoxications may arise from eating apricot kernels, chokecherries and other stone fruit kernels with high concentrations of cyanogenic glycosides. Inadequately prepared cassava, when constituting the major part of the diet, may be hazardous (WHO, 2001). Cyanide causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle. Cyanide can inhibit several other metalloenzymes most of which contain iron, copper, or molybdenum (e.g. alkaline phosphatase) as well as enzymes containing Schiff base intermediates (e.g. 2-keto-4-hydroxyglutarate aldolase). Hydrogen cyanide will reduce the energy availability in all cells but its effects will be most immediate on the respiratory system and heart.

Previous studies with laboratory animals have demonstrated that exposure to acutely toxic doses of cyanide can cause nerve damage and disturbances of thyroid function (Ferraro, 1933; Hurst, 1940; Ibrahim et.al., 1963; Lessell, 1971). In those animal studies, however, the levels of cyanide necessary to produce lesions were near or within the lethal range. The effects of subchronic administration of cyanide are less clear. In a 2-year feed study in which rats were administered feed containing hydrogen cyanide at concentrations up to 300ppm, there were no increases in mortality, decreases in body weight gain, hematologic changes, or gross or histologic lesions in any tissue of any exposure group (Howard and Hanzal, 1955). In rats administered feed containing 1,500- ppm potassium cyanide for 11.5months (Philbrick et al.1979) observed decreases in body weight gain, decreases in thyroid function that were not accompanied by discernible histologic lesions and modest myelin degeneration in spinal cord white matter. Philbrick and co-workers also found evidence of decreased thyroid function and vacuolation of nervous tissue in rats fed a diet containing 2,500ppm potassium thiocyanate for 11.5months. This concentration caused no change in body weight gain. The studies by (Philbrick et al 1979) included only one dose level of each compound and no data verifying compound levels in the feed were presented; therefore, the significance of the results is difficult to assess. Nevertheless, the literature data do indicate that repeated exposure to doses of cyanide that are marginally toxic is capable of producing thyroid gland and nervous system changes in rodents.

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The neurologic and thyroid gland lesions attributed to subchronic poisoning by cyanide and cyanogenic compounds in humans (Hardy et. al., 1950; Wilson, 1965; Osuntokun, 1968; Osuntokun et. al., 1970; El Ghawabi et.al., 1975; Towill et. al., 1978) are similar to those described in experimental animals receiving repeated high doses of cyanide (Ferraro, 1933; Hurst, 1940; Ibrahim et. al., 1963; Lessell, 1971). However, few quantitative exposure data are available in these cases of human poisoning. In studies where disturbances of thyroid function or goitre were seen in humans, exposure to cyanide vapours was described as "frequent" or "almost constant," and the thyroid gland effects were accompanied by signs of acute cyanide poisoning, including headache, dizziness, and difficulty in breathing. No studies describing thyroid gland effects in humans exposed to low, non acute toxic levels of cyanide were found in the literature. Visual and other neurological disturbances attributed to cyanide generally occur in individuals exposed to relatively high levels of cyanide or cyanogenic compounds (e.g., tropical neuropathies in persons consuming cassava as a significant percentage of the diet; tobacco amblyopia in persons who smoke) or individuals with inborn deficiencies in cyanide detoxification (e.g., optical neuropathy in persons with Leber's hereditary optic atrophy). Thus, while there is strong evidence for neuro toxic and thyrotoxic effects of cyanide in humans, these effects may represent high-dose phenomena, and the risk from low-level chronic exposures may be less. Alternatively, although humans are generally considered to be less sensitive than rodents to the acute effects of cyanide intoxication (McNamara, 1976), it is possible that humans may be more sensitive to the neurologic and thyroid gland effects.

It is not easy to determine what the lethal doses of cyanide to man is. The lethal dose for an adult depends on body weight and nutritional status. Human cyanide poisoning is associated with a mortality rate of 95% (Borowitz et. al., 1992). Taken orally the fatal dose of HCN to adult is estimated at 50-100 mg, and for potassium cyanide (KCN), about 150-250 mg (Ballantyne, 1974). However, victims ingesting as much as 3g of KCN have been saved with immediate therapy (Vanhleijst, 1987). Inhalation of HCN at a concentration of 270 ppm (approximately 0.3 mg HCN per litre) will be immediately fatal. Victims having a blood cyanide level of 2.5-3.0 µg/ml frequently succumb to respiratory cessation within 20-30 min of exposure or may survive even up to 3 hr (Ballantyne, 1974). The morbidity or mortality depends upon the magnitude of poisoning, which varies with the dose and form of cyanide and the route of poisoning (Vanhleijst et. al., 1987). If the hydrogen cyanide is somewhere between 30 and 210mg and it exceeds the limit an individual is able to detoxify / tolerate, death

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may occur due to cyanide poisoning depending upon the magnitude of exposure, time and intensity. Various non-specific signs and symptoms like headache, dizziness, nausea, vomiting, confusion, coma and incontinence of faeces and urine occur (Ballantyne, 1974). Physiologically a series of events like dyspnoea, incoordination of movement, cardiac irregularities, convulsive seizures, coma and respiratory failure may occur leading to death (Baskin et al., 1992). Pathologically no particular lesions can delineate cyanide toxicity, albeit animal experiments indicate that the lesions are principally in the central nervous system, predominantly necrosis in the white matter (Way, 1984). Probably the most widespread pathologic condition attributed to chronic cyanide poisoning is tropic ataxic neuropathy following cassava consumption (Osuntokun, 1980). Smaller, non-fatal amounts of cyanide cause acute intoxication with symptoms of rapid respiration drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting and diarrhoea.

2.4 COMPARATIVE KINETICS AND METABOLISM OF CYANIDE IN LABORATORY ANIMALS AND HUMANS

2.4.1 Absorption

Hydrogen cyanide and other cyanide salts, is readily absorbed following inhalation, oral, and dermal exposure. Following exposure to cyanide in the atmosphere, toxic amounts of cyanide are absorbed with great rapidity through the bronchial mucosa and alveoli (ATSDR, 1997). Humans retained 58% of the hydrogen cyanide in the lungs after inhaling the gas through normal breathing (Landahl and Herrmann, 1950; ATSDR, 1997). Alkali metal cyanides are rapidly absorbed from the gastrointestinal tract. The presence of food in the gut, the pH of the gut, and the lipid solubility of the cyanide compound affect absorption. Gastrointestinal absorption of inorganic cyanide salts is slower than pulmonary absorption, and the onset of symptoms is delayed and the severity of symptoms diminished compared with inhalation (WHO, 2004). When simple cyanide salts such as potassium and sodium cyanide are ingested, free cyanide ion can rapidly bind hydrogen ion to form hydrogen cyanide in the highly acidic medium of the stomach. Essentially all cyanide ingested as cyanide salts will form hydrogen cyanide and will be quickly absorbed. However, after oral intake, only part of the dose reaches the blood due to first-pass metabolism by the liver (IPCS IOC, 2004). Cyanides are well absorbed via the gastrointestinal tract or skin and rapidly absorbed via the respiratory tract. Once absorbed, cyanide is rapidly and ubiquitously distributed throughout the body, although the highest levels are typically found in the liver, lungs, blood, and brain. There is no accumulation of cyanide in the blood or tissues following chronic or repeated exposure.

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Liquid cyanide compounds are easily absorbed through intact skin upon direct contact due to their lipid solubility and rapid epidermal penetration. Skin absorption of vapours of hydrogen cyanide is also possible when the air concentrations are high (WHO, 2004). The amount and rate of absorption of cyanides from aqueous solutions or atmospheric hydrogen cyanide depend upon the presence of moisture in the skin, concentration and pH of the solution, the surface area of contact, and the duration of contact (Dugard, 1987). *In vitro* studies with human skin have shown that penetration of sodium cyanide in aqueous solution through skin decreases with increasing pH (increasing dissociation), reflecting the more rapid absorption of the un-dissociated hydrogen cyanide. The permeability constant measured for the cyanide ion in aqueous solution was 3.5×10^{-4} cm/h, and that calculated for hydrogen cyanide was 1×10^{1} cm/h (Dugard, 1987).

2.4.2 Distribution

Hydrogen cyanide has a pK_a of 9.22; thus, at physiological pH (about pH 7.4), hydrocyanic acid is distributed in the body as hydrogen cyanide and is not present as the free cyanide ion. Hence, the form of cyanide to which exposure occurs, the salt or the free acid, does not influence distribution, metabolism, or excretion from the body (ECETOC, 2004). Inhaled or percutaneously absorbed hydrogen cyanide passes immediately into the systemic circulation. The distribution of cyanide to the various tissues is rapid and fairly uniform. Somewhat higher levels are generally found in the liver, lungs, blood, and brain. The tissue levels of hydrogen cyanide were 0.75, 0.42, 0.41, 0.33, and 0.32 mg/100 g of tissue in lung, heart, blood, kidney, and brain, respectively, in a man who died following inhalation exposure to hydrogen cyanide gas (Gettler and Baine, 1938; Ballantyne, 1983; ATSDR, 1997; ECETOC, 2004). In contrast, high proportions of ingested sodium and potassium cyanide will pass through the liver and are detoxified by the first-pass effect. The major portion of cyanide in blood is sequestered in the erythrocytes, and a relatively small proportion is transported via the plasma to target organs. Cyanide is concentrated in red blood cells at a red blood cell to plasma ratio of 199:1; levels in plasma reflect tissue levels better than levels in whole blood or erythrocytes. Small but significant levels of cyanide are found in normal blood plasma (<1.10 µg/litre) and other tissues (<0.5 mg cyanide/kg) of humans without known occupational cyanide exposure (Eckstein & Klendshoj, 1954). These levels are related mostly to exposure to cyanogenic food, vitamin B_{12}, and tobacco smoke. A detailed survey of normal plasma cyanide levels in 10 cases showed a maximum level of 106 µg/litre, with a mean of 48 µg/litre (Eckstein &

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Klendshoj, 1954). After cessation of exposure, plasma cyanide levels tend to return to normal within 4–8 h (Feldstein & Klendshoj, 1954; Ansell & Lewis, 1970).

In rats dosed by gavage, highest concentrations of cyanide were found in the liver, followed by the lungs and blood (Yamamoto, 1990). After inhalation exposure, the highest concentrations of cyanide in rats were found in the lungs, followed by the blood and liver. There is a cumulative effect of exposure to thiocyanate (from the breakdown of cyanogenic glycosides in food plants), resulting in thyroid toxicity, including goiter and cretinism (Nahrstedt, 1993). A number of illustrative levels of cyanide in organs and blood after oral intake in humans (Ansell and Lewis, 1970; ATSDR, 1997) and rabbits (Ballantyne, 1983a) have been reported. For a given exposure route, whole blood and serum cyanide levels are quite similar for different species (Ballantyne, 1983).

2.4.3 Metabolism and Excretion

Although cyanide can interact with substances such as methaemoglobin in the bloodstream, the majority of cyanide metabolism occurs within the tissues. Cyanide is metabolized in mammalian systems by one major route and several minor routes. The major route of metabolism for hydrogen cyanide and cyanides is detoxification in the liver by the mitochondrial enzyme rhodanese, which catalyses the transfer of the sulfane sulfur of thiosulfate to the cyanide ion to form thiocyanate (Figure 3) (Williams, 1959; Ansell and Lewis, 1970). This route detoxifies about 80% of cyanide. The rate-limiting step is the amount of thiosulfate. While rhodanese is present in the mitochondria of all tissues, the species and tissue distributions of rhodanese are highly variable. In general, the highest concentrations of rhodanese are found in the liver, kidney, brain, and muscle, but the supply of thiosulfate is limited (Aminlari et al., 1994). Rhodanese is present in rat nasal mucosal tissues, particularly in the olfactory region, at a 7-fold higher concentration (on a per milligram of mitochondrial protein basis) than in the liver (Dahl, 1989). Dogs have a lower overall activity of rhodanese than monkeys, rats, and rabbits (ATSDR, 1997).

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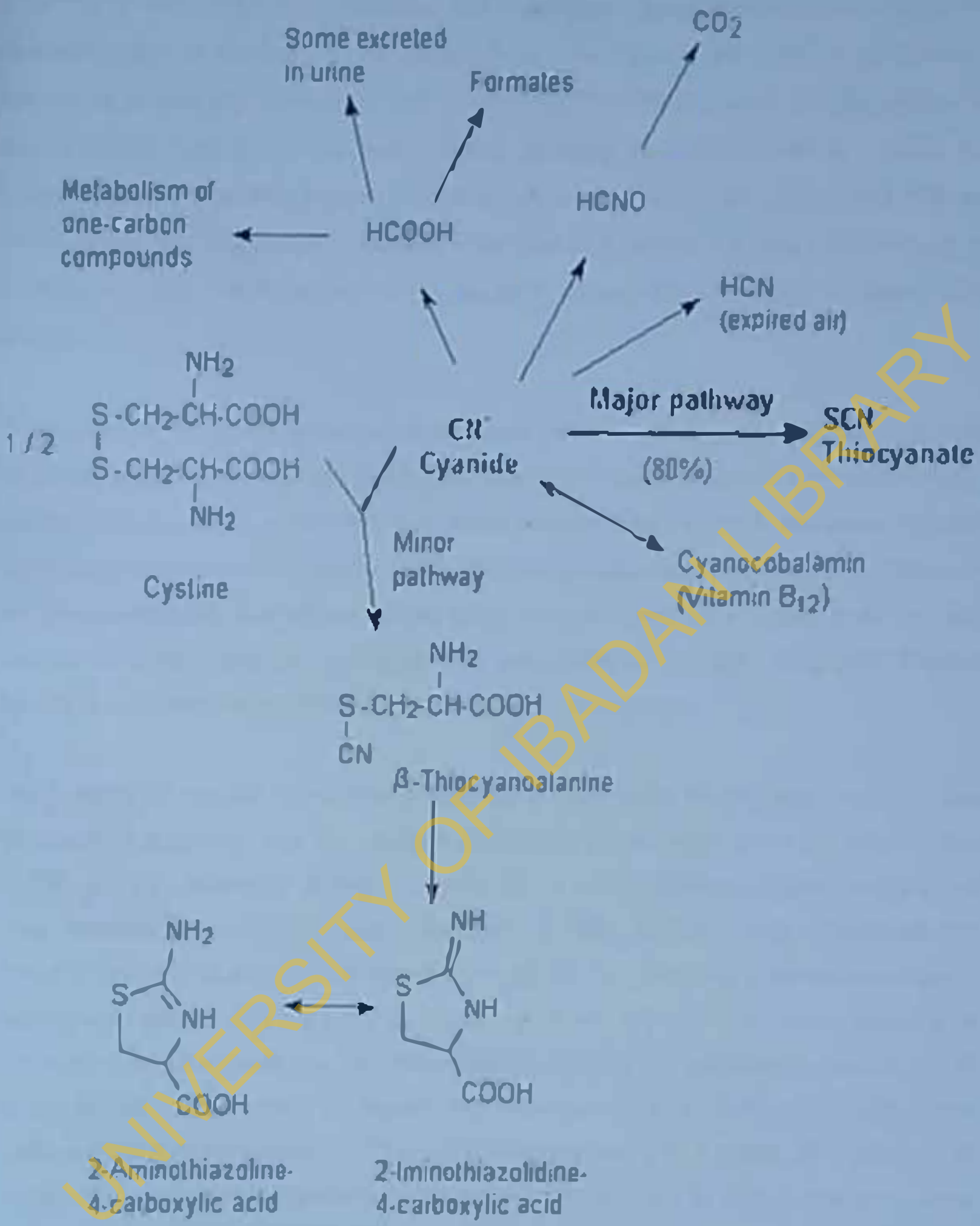

Figure 2.3: Basic processes involved in the metabolism of cyanide (WHO, 1997)

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A number of other sulfur transferases can also metabolize cyanide, and albumin, which carries elemental sulfur in the body in the sulfane form, can assist in the catalysis of cyanide to thiocyanate as well (Sylvester et al., 1983; Westley et al., 1983). Cyanide and thiocyanate can also be metabolized by several minor routes, including the combination of cyanide with hydroxycobalamin (vitamin B_{12a}) to yield cyanocobalamin (vitamin B_{12}) (Boxer and Richards, 1952) and the non-enzymatic combination of cyanide with cystine, forming 2-iminothiazoline-4-carboxylic acid, which appears to be excreted without further change (Rieders, 1971) (Figure 2.3).

In studies with rats orally administered potassium cyanide and maintained for up to 4 weeks on either a balanced diet or a diet lacking the sulfur amino acids L-cystine and L-methionine, a strongly positive linear relationship was found between blood cyanide and plasma cyanate (OCN^-) concentration (Tor-Agbidye et al., 1999). It was suggested that in Africa, where there are protein-deficient populations whose levels of sulfur-containing amino acids are low, cyanide (from prolonged use of cassava) may conceivably be converted to cyanate, which is known to cause neurodegenerative disease in humans and animals.

While absorbed cyanide is principally excreted as thiocyanate in the urine, traces of free hydrogen cyanide may also be excreted unchanged in the lungs, saliva, sweat, or urine (Hartung, 1982), as carbon dioxide in expired air, or as *beta*-thiocyanoalanine in saliva and sweat (Friedberg and Schwartzkopf, 1969; Hartung, 1982; IPCS, 1993). Thiocyanate was found in the urine of non-exposed people at average concentrations of 2.16-mg/litre urine for non-smokers and 3.2-mg/litre urine for smokers (Chandra et al., 1980). Urinary excretion of thiocyanate was monitored in a man after ingestion of about 3–5 g potassium cyanide (15–25 mg cyanide/kg body weight) (Liebowitz and Schwartz, 1948; ATSDR, 1997). The results indicated that the patient excreted 237 mg of thiocyanate over a 72-h period. This quantity was substantially more than the normal average amount of thiocyanate in urine, which varies from 0.85 to 14 mg/24 h (ATSDR, 1997).

The limiting factor in cyanide metabolism is the low concentration of the sulfur-containing substrates in the body — primarily thiosulfate, but also cystine and cysteine. The rate of spontaneous detoxification of cyanide in humans is about 1 µg/kg body weight per minute (Schultz and Roth et al., 1982), which is considerably slower than in small rodents (Schubert and Brill, 1968) or dogs (Lawrence, 1947).

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2.5 POTENTIAL HEALTH EFFECTS IN HUMANS OF POTASSIUM CYANIDE

2.5.1 Effects of Short-Term (Acute) Exposure

Inhalation:

Potassium cyanide is a solid, which does not form a vapour at room temperature. However, inhalation of potassium cyanide can occur following exposure to the dust and to mists or vapours from heated or misted solutions. In general, dusts or mists can be very irritating to the nose and throat. More importantly, potassium cyanide releases hydrogen cyanide when combined with water or acid. Hydrogen cyanide is an extremely toxic gas, which causes death at very low concentrations. It is a rapidly absorbed and fast-acting poison, which poses a very serious inhalation hazard. The odour threshold of hydrogen cyanide is very low (0.6–1.5 ppm), but it does not provide a reliable warning of exposure. Some people (up to 20% of the population) are unable to smell cyanide, even at highly toxic concentrations (ATSDR, 1997). The early symptoms of cyanide poisoning may include anxiety and excitement, weakness, headache, nausea, vomiting, metallic taste, chest tightness, facial flushing, drowsiness, dizziness, irritation of the eyes, nose and throat, rapid breathing, a rise in blood pressure and a decrease in pulse. Laboured breathing, falling blood pressure, rapid, weak irregular heartbeat, unconsciousness, and convulsions follow these symptoms. In severe cases, cardiovascular collapse, shock, and fluid accumulation in the lungs (pulmonary edema) are followed by death. With massive doses, many of the signs and symptoms may not be seen, and there is a rapid onset of poisoning with convulsions, collapse and death (Ballantyne et al., 1983).

A characteristic sign of cyanide poisoning is the bright red colour of blood, which may result in red skin colour (Gosselin et. al., 1984). There are many reports of cyanide poisoning from accidental, suicidal and homicidal exposure to HCN or its salts (most commonly potassium or sodium cyanide). The majority of people who survive short-term cyanide poisoning do not have long-lasting effects. However, depending on the degree of exposure, there may be enduring effects from low oxygen, including impaired memory and mathematical abilities, personality changes, and altered control and coordination of movement (Hall et. al., 1986).

Skin Contact:

Potassium cyanide is very toxic if absorbed through the skin. Skin contact with potassium cyanide solutions can cause symptoms similar to those described under "Inhalation" above. Potassium cyanide solutions are expected to be corrosive, based on pH. Corrosive materials

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can cause severe skin burns with blistering, permanent scarring and, in severe cases death. No conclusions can be drawn from a case report that describes an electroplater and metal worker who developed a unique neuro-behavioural disorder, diagnosed as an acute psychosis, following a significant short-term exposure to cyanide. (He was splashed in the face by an unspecified cyanide compound.) This person also had significant long-term exposure to several metals, organic solvents and electroplating chemicals (Kales et. al., 1997).

Eye Contact:

Potassium cyanide is very toxic if absorbed through the eye. Eye contact can cause symptoms as described under "Inhalation" above. Potassium cyanide solutions are expected to be corrosive, based on pH. Corrosive materials can cause very severe eye irritation and, in some cases, permanent damage to vision, including blindness.

Ingestion:

Potassium cyanide is very toxic if ingested. It is rapidly absorbed through the digestive tract resulting in symptoms as described under "Inhalation" above. Immediately following ingestion, a bitter, acrid, burning taste may be noted, followed by constriction or numbness in the throat. There is rapid ventilation and shortness of breath, the stomach lining is irritated and nausea and vomiting may occur. Then unconsciousness, convulsions, muscular contraction of the jaw, rapid and irregular pulse, gasping, paralysis and death may occur (Hasu et. al., 1985). In humans, the average lethal dose of hydrogen cyanide is estimated to be 60-90 mg (Gosselin et. al., 1984). A few cases of Parkinsonism (a syndrome characterized by decreased mobility, muscular rigidity, and tremor) have been reported in survivors of acute cyanide poisoning. All case reports involved non occupational exposure to high oral doses (where specified) (Grandas et. al., 1989). Ingestion is not a typical route for occupational exposure. If the hydrogen cyanide exceeds the limit an individual is able to detoxify/tolerate, death may occur due to cyanide poisoning. The acute oral lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg bodyweight. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man. Data on the oral lethal dose of cyanide for man in four cases of suicide, calculated from the amount of hydrogen cyanide absorbed in the body at the time of death, and from the amount of hydrogen cyanide found in the digestive tract, differed considerably and corresponded to doses of 0.58-22 mg/kg body weight (WHO, 1965).

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Although acute cassava poisoning — sometimes leading to the death of whole families — has been occasionally reported after the consumption of inadequately processed cassava (Osuntokun, 1981; Cliff and Countinho, 1995).

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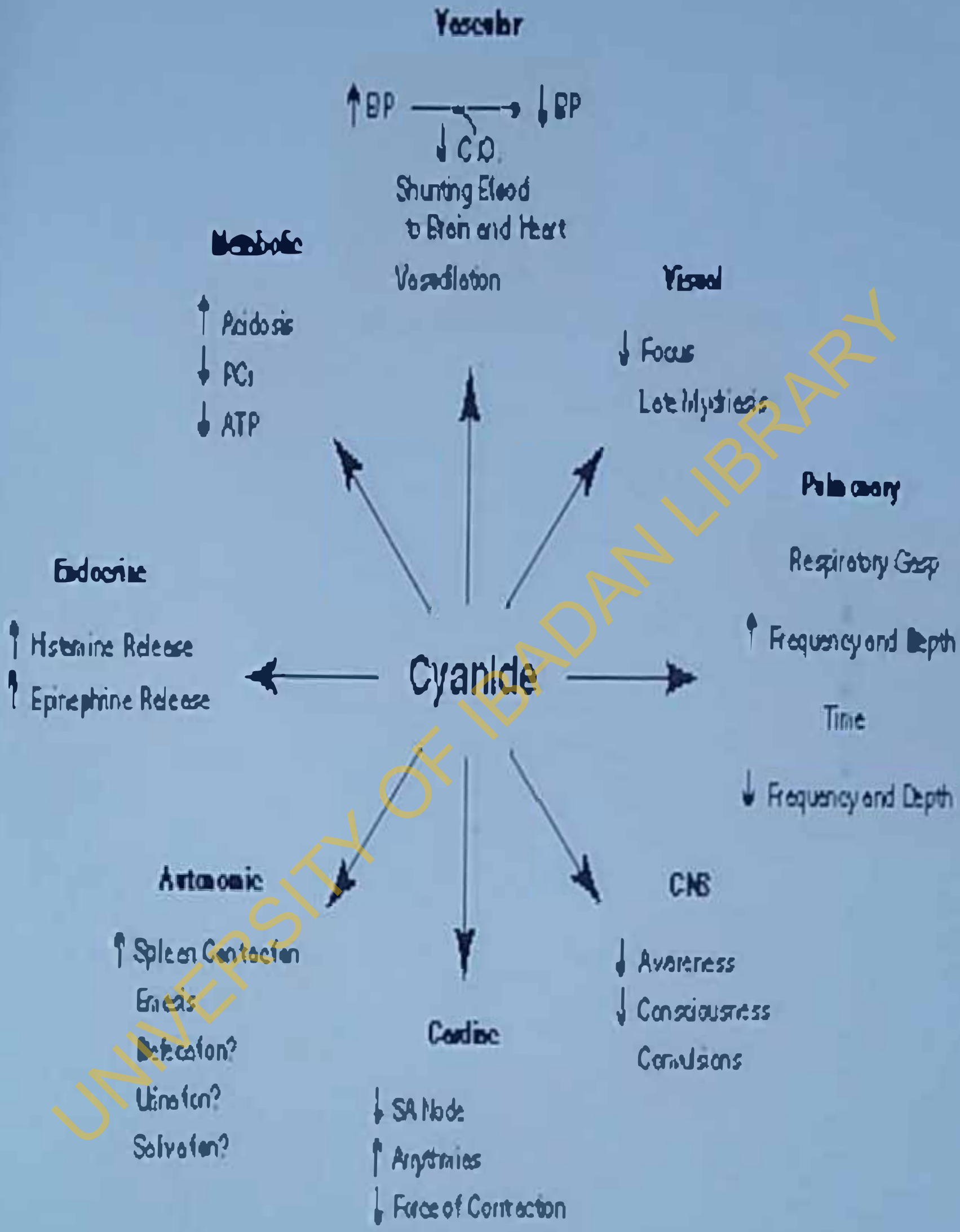

Figure 2.4: Cyanide toxicity pathways

Baskin et al 1998

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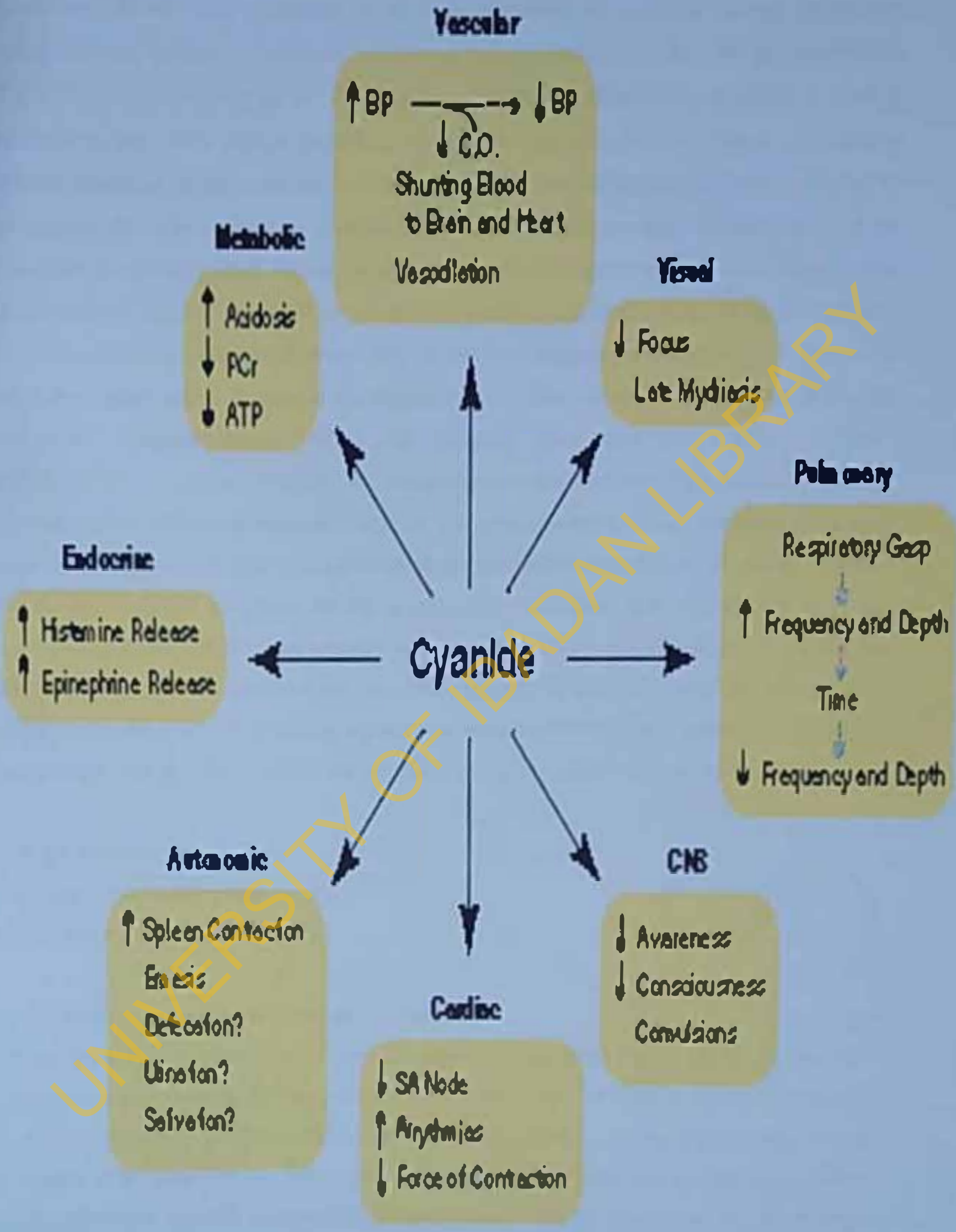

Figure 2.4: Cyanide toxicity pathways

Baskin et. al. 1998

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Cyanide can affect many functions in the body, including the vascular, visual, pulmonary, central nervous, cardiac, autonomic, endocrine, and metabolic systems. The toxico-dynamic effects can vary depending on the dose, route and speed of administration, chemical form of the cyanide, and other factors including the gender, age, weight, stress level, and general physical condition of the recipient (Baskin et. al. 1998). Proceeding clockwise from the top of the diagram: *Vascular* effects for cyanide can include an initial transient increase, followed by a decrease, in cardiac output. Blood pressure falls as the cardiac inotropic effect decreases and as vasodilation occurs. *Visual* effects can include a decrease in the capacity to focus, with late-onset mydriasis secondary to hypoxia. One of the first *pulmonary* effects from cyanide is a respiratory gasp, which is caused by stimulation of chemoreceptor bodies near the aortic bifurcation. Hyperventilation follows this response. Over time (the response is dose-dependent, but seconds to minutes), the frequency and depth of breathing diminish. *Central nervous system* effects initially manifest as decreased awareness and increased release of enkephalins followed by loss of consciousness and convulsions (Baskin et. al., 1998). *Cardiac* effects after cyanide exposure are an increase in heart rate, then a decrease; both are accompanied by arrhythmias and negative inotropy. Cyanide produces a number of *autonomic nervous system* effects, based on the route and dose of the agent. Cyanide can also produce multiple *endocrine* effects including epinephrine and histamine release, and *metabolic* actions that decrease energy production by the inhibition of the use of cytochrome oxidase.

PCr: phosphocreatine

ATP: adenosine triphosphate

C.O.: cardiac output

2.5.2 Effects of Long-Term (Chronic) Exposure

Several human population studies have evaluated the potential health effects of long-term cyanide exposure. In general, these studies are limited by factors such as the small number of employees evaluated and the possibility of concurrent exposure to other potentially harmful chemicals (particularly in the electroplating industry). In addition, few studies report reliable measurements of cyanide exposures and even when airborne concentrations are reported, exposure may also have occurred by skin absorption. Despite these limitations, the available evidence suggests that long-term occupational cyanide exposure may be associated with harmful effects in the thyroid gland and the nervous system. Long-term exposure to cyanide

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also occurs from smoking, eating foods containing cyanogenic glycosides and infection with cyanide-producing bacteria (Wilson, 1987).

Nervous System:

Limited information suggests that long-term exposure to cyanides may be associated with harmful effects on the nervous system. Some of the symptoms observed are non-specific (e.g. headaches) and could be associated with many causes. Nevertheless, there does seem to be an association between some nervous system symptoms and cyanide exposure. Thirty-six male, non-smoking employees were exposed for 5-15 years to 4.2-12.4 ppm cyanide from electroplating baths containing sodium and copper cyanide. Nervous system symptoms were, in order of frequency, headache, weakness, changes in taste and smell, visual difficulties, and nervous instability. Two employees experienced psychotic episodes, which they recovered from within 36–48 hours following removal from the area of exposure (El Ghawabi et al., 1975). Fifty-six male employees were exposed to hydrogen cyanide (concentrations not reported) while engaged in case hardening and electroplating for 2-20 years. A significant increase in impairment of memory, visual ability, visual learning and psychomotor ability was observed in exposed employees, compared to 33 matched controls. Headaches were more frequently reported in exposed workers (Kumar et al., 1992).

Thirty-six employees were exposed to hydrogen and sodium cyanide in a silver reclaiming factory by inhalation (15 ppm, 24-hour average concentration), skin contact and possibly oral exposure. An employee died of acute cyanide poisoning and the plant was closed for 7 months before the study was carried out. An overall exposure index was calculated based on job category, frequency of handling cyanide and ingesting food or drink in the production areas. Nervous system symptoms, which had a significant positive correlation with exposure, were numbness or tingling (paresthesia) of the extremities, easy fatigue and a symptom complex including headache, dizziness, and fainting (Blanc et al., 1985). Neuropathies in people living in tropical areas with a diet high in cassava, a root rich in cyanogenic glycosides, have previously been attributed to cyanide.(ATSDR, 1997) However, this diet is also high in scopoletin, a coumarin compound, which is believed to be responsible for some of the neurotoxic effects (Obidoa et al., 1991).

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Lungs/Respiratory System:

Two limited studies suggest that long-term cyanide exposure may be associated with laboured breathing. An increased incidence of effort-induced, laboured breathing was observed in 36 male, non-smoking employees exposed for 5-15 years to 4.2-12.4 ppm cyanide from electroplating baths containing sodium and copper cyanide (El Ghawabi et. al., 1975). An association between laboured breathing and cyanide exposure was also observed in 36 employees exposed to hydrogen and sodium cyanide in a silver-reclaiming factory, by inhalation (15 ppm, 24-hour average concentration), skin contact and possibly oral exposure. An employee had died of acute cyanide poisoning and the plant was closed for 7 months before the study was carried out. An overall exposure index was calculated based on job category, frequency of handling cyanide and ingesting food or drink in the production areas (Blanc et. al., 1985).

Skin:

An association between development of a skin rash and cyanide exposure was also observed in 36 employees exposed to hydrogen and sodium cyanide in a silver-reclaiming factory, by inhalation (15 ppm, 24-hour average concentration), skin contact and possibly oral exposure. An employee had died of acute cyanide poisoning and the plant was closed for 7 months before the study was carried out (Blanc et. al., 1985).

Digestive System:

An increased incidence of nausea and/or vomiting was reported in two studies that evaluated employees with long-term exposure to cyanide concentrations up to 15 ppm (with possible concurrent ingestion and skin contact) (El Ghawabi et. al., 1975).

Eyes/Vision:

Eye irritation was reported in 3 limited studies involving electroplating workers. Exposures, when specified, ranged from 4.2-15 ppm cyanide (Kumar et. al., 1992). However, it is not possible to draw any specific conclusions about the eye irritation potential of long-term cyanide exposure, because electroplating workers are exposed to many chemicals that are irritating to the eyes (ATSDR, 1997). Degeneration of the optic nerve and part of the retina (the macula) is found in people living in tropical areas with a diet high in cassava, a root rich in cyanogenic glycosides (Wilson, 1987). In some cases, these effects have been attributed to

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cyanide exposure (ATSDR, 1997). However, this diet is also high in scopoletin, a coumarin compound, which is believed to be responsible for some of these effects (Obidoa et al., 1991.)

Blood/Blood Forming System:

There is very limited information that long-term exposure to cyanide is associated with harmful effects on the blood. Blood chemistry changes (increased white blood cells and red blood cell sedimentation rate, and decreased hemoglobin level) was observed in 31 employees exposed to unspecified concentrations of hydrogen cyanide, while engaged in case hardening and electroplating for 2-20 years (Kumar et al., 1992). Statistical analysis of the results was not conducted. Blood chemistry changes (increased hemoglobin and lymphocyte counts and red blood cell damage) were observed in 36 male, non-smoking employees exposed for 5-15 years to 4.2-12.4 ppm cyanide during electroplating operations (El Ghawabi et. al., 1975). However, exposure to copper, an agent known to have toxic effects on blood also occurred. Changes in white blood cell enzyme activity were noted in 43 employees exposed to an average concentration of 0.23 ppm hydrogen cyanide for 0.25-16 years (average 5.4 years) during metal coating operations (Dinca et al., 1972).

Endocrine System:

Evidence from human and animal studies indicates that long-term exposure to cyanide can result in impaired thyroid function and enlargement of the thyroid (goiter). Thiocyanate, the main metabolite of cyanide, is believed to cause these effects by inhibiting the uptake of iodine by the thyroid (Banerjee et. al., 1997). Findings consistent with impaired thyroid function were observed in 35 male employees, all non-smokers, who were exposed to cyanide salts for at least 5 years while working with an electroplating process. Cyanide concentrations were not reported (Banerjee et. al., 1997). Mild to moderate thyroid enlargement was observed in 20/36 male electroplating workers, who were exposed to 4.2-12.4 ppm cyanide for 5-15 years. Measurement of radioactive iodine uptake showed a significantly higher iodine uptake in the exposed workers than for the control group (El Ghawabi et. al., 1975). The health of 36 employees exposed to hydrogen and sodium cyanide in a silver-reclaiming factory was assessed. Inhalation (15 ppm, 24-hour average concentration), skin contact and possibly oral exposure had occurred. An employee died of acute cyanide poisoning and the plant was closed for 7 months before the study was carried out. An overall exposure index was calculated based on job category, frequency of handling cyanide and ingesting food or drink in the production area. In tests done 7-30 months after the last exposure, the thyroid-stimulating hormone was

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significantly higher in high exposure index employees, compared to the mean laboratory control value. However, thyroxine levels were normal and no thyroid enlargement was found (Blanc et al., 1985). Limited animal information suggests that long-term exposure to cyanide compounds may harm the thyroid gland.

Carcinogenicity:

There is no human or animal information available. The International Agency for Research on Cancer (IARC) has not evaluated the carcinogenicity of this chemical. The American Conference of Governmental Industrial Hygienists (ACGIH) has not assigned a carcinogenicity designation to this chemical. The US National Toxicology Program (NTP) has not listed this chemical in its report on carcinogens.

Teratogenicity and Embryotoxicity:

There is no human information available. The limited animal information available suggests that potassium cyanide is not a developmental toxin.

Reproductive Toxicity:

There is no human information available. In an animal study, changes suggestive of reproductive effects were observed in rats and mice. However, fertility was not evaluated.

Mutagenicity:

There is no human information available. The available evidence does not indicate that potassium cyanide is mutagenic. Two tests using live mice were negative. Both positive and negative results have been obtained in short-term tests using mammalian cells and bacteria.

Toxicologically Synergistic Materials:

Co-exposure to hydrogen cyanide and 5% carbon dioxide (not lethal by itself) resulted in an increase in the lethality of hydrogen cyanide (ATSDR, 1997). Oral pre-treatment of guinea pigs with ascorbate enhanced the toxic effects of oral administration of potassium cyanide. It was suggested that the ascorbate interfered with the reaction to detoxify cyanide (Basu, 1983).

Potential for Accumulation:

Cyanide does not accumulate. The most important route for detoxification is by a mitochondrial enzyme, rhodanese, which adds sulfur to the cyanide ion to form thiocyanate. Thiocyanate is less toxic, and is excreted in the urine (Basu et al., 1984). This enzyme is

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widely distributed in the tissues, but has its greatest activity in the liver. The body has a large capacity to detoxify cyanide but the reaction is dependent on an adequate supply of sulfur (Gosselin et. al., 1984). The maximum detoxification rate for humans is 0.6-0.9 micrograms/kg body weight/minute, which is considerably lower than for lab rodents or dogs. Most absorbed cyanide is excreted in the urine as thiocyanate, but small amounts are eliminated in exhaled air and urine as hydrogen cyanide, carbon dioxide and other metabolic products. The average half time for excretion of thiocyanate has been reported to be 2.7 days in healthy volunteers.

Health Comments:

The cyanide ion binds with iron ions in the enzyme cytochrome oxidase, which prevents body cells from using oxygen. Thus, cyanide impairs the body's ability to use oxygen and the primary target organs for acute cyanide poisoning are the central nervous system and the heart (ATSDR, 1997) .Cyanides also inhibit other enzyme systems, especially those containing iron or copper, which contributes to the symptoms observed (Beasley, 1998).

2.5.3 Long-Term Studies and Cyanide Diseases

Konzo

'Konzo' is a local Zairean term for a disease first described in 1938 in the Democratic Republic of Congo (formally Zaire) by Trolli in 1938, but has also been observed in Mozambique Tanzania, Central African Republic and Cameroon (Ministry of Health, Mozambique, 1984; Howlett et al., 1990; Tylleskar et al., 1992, 1994; Lantrum et al, 1998; Ernesto et al., 2002). Konzo is an upper motor neuron disease characterised by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age. Severe cases have a spastic toe-scissor gait or patients will not be able to walk at all, and the arms and speech may also be affected. A long-term follow-up of konzo patients showed that the neurological signs in konzo patients remained constant, however, functional improvement may occur (Cliff and Nicala, 1997). High urinary thiocyanate concentrations and presence of ankle clonus are also observed. In all reports of epidemics, konzo has been associated with high and sustained cyanogens intake at sub-lethal concentrations from cassava or cassava flour in combination with a low intake of sulphur amino acids.

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Tropical Ataxic Neuropathy (TAN)

TAN is used to describe several neurological syndromes attributed to toxico-nutritional causes. The syndromes grouped as TAN can differ widely in clinical presentation, natural history and response to treatment. TAN has occurred mainly in Africa, particularly Nigeria. The main clinical features of some of the syndromes have included: sore tongue, angular stomatitis, skin desquamations, optical atrophy, neuro-sensory deafness and sensory gait ataxia (in Oluwole et al. 2000). The cause is attributed to dietary cyanide exposure from the chronic monotonous consumption of foods processed from cassava. The onset of TAN is usually slow over months or years and the mean age of people affected by TAN is greater than 10 years. TAN affects males and females in all age groups equally.

Goitre and cretinism

Studies in African countries such as Zaire have established that goitre and cretinism due to iodine deficiency can be considerably aggravated by a continuous dietary cyanide exposure from insufficiently processed cassava. This effect is caused by thiocyanate, which is similar in size to the iodine molecule and interferes with uptake of iodine into the thyroid gland. High thiocyanate levels, which can occur after exposure to cyanide from cassava, can only affect the gland when the iodine intake is below 100micrograms/day, which is regarded minimal for normal function. Populations with very low iodine and high thiocyanate level from consumption of cassava show severe endemic goitre, but this decrease with iodine supplementation (reviewed by Rosling, 1987).

2.6 KINETICS OF CYANIDE AND HEALTH EFFECTS IN HUMAN

Cyanide is produced in the human body and exhaled in extremely low concentrations with each breath. It is acutely toxic to humans. Liquid or gaseous Hydrogen cyanide and alkali salts of cyanide can enter the body through inhalation, ingestion or absorption through the eyes and skin. The rate of skin absorption is enhanced when the skin is cut, abraided or moist; inhaled salts of cyanide are readily dissolved and absorbed upon contact with moist mucous membranes. The dose-effect curve of the acute effects in humans is steep. Whereas slight effects occur at exposure to hydrogen cyanide levels of 20-40 mg/m^3, 50-60 mg/m^3 can be tolerated without immediate or late effects for 20 min to 1 h, 120-150 mg/m^3 is dangerous to life and may lead to death after 0.5-1 h, 150 mg/m^3 is likely to be fatal within 30 min, 200 mg/m^3 is likely to be fatal after 10 min. and 300 mg/m^3 is immediately fatal. It should be

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emphasized that this represents crude average exposure estimates, based on various studies (DICOS, 2002).

The effects of acute cyanide exposure are dominated by central nervous system and cardiovascular disturbances (ATSDR, 1991). Typical signs of acute cyanide poisoning include tachypnoea, headache, vertigo, lack of motor coordination, weak pulse, cardiac arrhythmias, vomiting, stupor, convulsions, and coma (Ballantyne, 1983; Way et al., 1984). Pathological findings may include tracheal congestion with haemorrhage, cerebral and pulmonary oedema, gastric erosions, and petechiae of the brain meninges and pericardium (Way, 1984). Sequelae of severe acute cyanide exposure may also include Parkinson-like syndromes and cardiovascular signs of delayed post-hypoxic myocardial lesions, as well as neuropsychiatric manifestations similar to those seen with post-hypoxic post-carbon monoxide encephalopathy (ATSDR, 1991). Dermal absorption of hydrogen cyanide is much slower than pulmonary absorption, and the amount and speed of absorption through human skin are dependent on the amount of skin moisture and duration of skin contact. The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure. Due to the variability of dose-response effects between individuals, the toxicity of a substance is typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC_{50} or LD_{50}). The LC_{50} for gaseous hydrogen cyanide is 100-300 parts per million. Inhalation of cyanide in this range results in death within 10-60 minutes, with death coming more quickly as the concentration increases. Inhalation of 2000 part per million hydrogen cyanide causes death within one minute. The LD_{50} for ingestion is 50-200 milligrams or 1-3 milligrams per kilogram of body weight, calculated as hydrogen cyanide. For contact with unabraded skin, the LD_{50} is 100 milligrams (as hydrogen cyanide) per kilogram of body weight. An average LD_{50} value for dermal exposure of 100 mg/kg body weight was estimated for humans (Rieders, 1971). Although the time, dose and manner of exposure may differ, the biochemical action of the cyanide is the same upon entering the body. Once in the blood stream, cyanide forms a stable complex with a form of cytochrome C oxidase, an enzyme that promotes the transfer of electrons in the mitochondria of cells during the synthesis of ATP. Without proper cytochrome oxidase function, cells cannot utilize the oxygen present in the blood stream, resulting in cytotoxic hypoxia or cellular asphyxiation. The lack of available oxygen causes a shift from aerobic to anaerobic metabolism, leading to the accumulation of lactate in the blood. The combined effect of the hypoxia and lactate acidosis is depressed in the central nervous system that can result in respiratory arrest and death. At higher lethal concentrations, cyanide-

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poisoning also affects other organs and system in the body including the heart. Initial symptoms of cyanide poisoning can occur from exposure to 20 to 40 ppm of gaseous hydrogen cyanide and may include headache, drowsiness, vertigo, weak and rapid pulse, deep and rapid breathing, nausea and vomiting. Convulsing, dilated pupils, clammy skin, a weaker and more rapid pulse and slower, shallower breathing can follow these symptoms (El Ghawabi et. al., 1975). Finally, the heartbeat becomes slow and irregular, body temperature falls, the lips, face and extremities take on a blue colour, the individual falls into a coma and death occurs (Hanung, 1982; USEPA, 1985). These symptoms can occur from sub lethal exposure to cyanide, but will diminish as the body detoxifies the poison and excretes it primarily as thiocyanate and 2 imino thiazoline -4 carboxilic acid with other minor metabolites (ATSDR, 1989).

Plants containing cyanoglycosides are the main source of cyanide exposure for individuals who are not occupationally exposed to the chemical. The general population is exposed to cyanides primarily by ingestion of food and water, and to a lesser degree, by inhalation. The cyanide content in unpolluted air averages 0.160–0.166 ppm (0.180– 0.187 mg/m^3). Cyanide levels in smoke from U.S. commercial cigarettes range from 10 to 400 µg/cigarette for mainstream (inhaled) smoke and from 0.006 to 0.27 µg/cigarette for side stream smoke. The cyanide content in 99.8% of public water systems using groundwater in the United States between 1993 and 1998 did not exceed the maximum concentration limit of 0.2 mg/L. Mean cyanide concentrations have been reported for some food products: cereal grains (0.002–0.45 µg/g), soy protein products (0.07–0.3 µg/g), canned unpitted fruits (0–4 µg/g), commercial fruit juices (1,900–1,600 µg/L), and U.S. lima beans (100–170 µg/g). There are no comprehensive data on the cyanide content of total diet samples in the United States, so it is not possible to estimate the average daily intake from foods. Cyanide is an extremely toxic and fast acting poison; however, it can be detoxified to a certain extent in the human body. In very small amounts, cyanide is a necessary requirement in the human diet as prosthetic group of cyanocobalamine (Vitamin B12). Cassava plant including the storage roots, contain linamarine and lotaustralin respectively, which break down upon disruption of plant cells to form hydrogen cyanide (Dietz et. al., 1994). Different neurological syndromes have been associated with exposure to cyanide. Dietary cyanide exposure from cassava roots combined with a low intake of the sulfur amino acids necessary for cyanide detoxification has been implicated in the causation of growth retardation and konzo, an upper motor neuron disease identified in Africa (Rosling, 1994). The lethal dose of cyanide for an adult depends on the

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bods' weight and nutritional status and this is somewhere between 30 and 210 mg of HCN. If the HCN exceeds the limit an individual is able to detoxify or tolerate, death may occur due to cyanide poisoning while smaller non- lotal amount of cyanide cause acute intoxication (USEPA, 1990). Since cassava can withstand drought, it is sometirnes a nutritionally strategic famine reserve crop in areas of unreliable rainfall, therefore the need to identify the nutritional problems associated with cassava dependency and the use of home grown vegetables in abating this toxicity especially in improperly processed cassava based foods. Vegetable species stems are noted for their rich conients of essential amino acids, vitamins and minerals. Fuither to their rich content of the mentioned nutrients, it is established that green vegetable leaves are the cheapest and most abundant source of proteins because of their ability to synthesize amino acids from a wide range of virtually available primary materials such as water, carbon dioxide, and atmospheric nitrogen (as in legumes) (Fasuyi, 2006).

The amount of cyanide in the blood that is likely to prove toxic is imprecise and depends heavily on when the sample is drawn in comparison to the time of exposure, the specific cyanide compound or cyanogenic compound involved, the route of exposure, treatmen provided before sampling (if any), and sample handling between collection and analysis. In adults, the blood cyanide level that is regarded as "toxic" is generally considered to be ≥ 1 mg/L (39 µmol/L), and the "fatal" level is generally considered to exceed 2.6 to 3 mg/L (100-115 µmol/L) (Yeaoh et. al., 2004). Inhalation of Fire Smoke approximately one fourth of the 4000 fire and burn-related deaths each year in the United States occur in children youngerthan 15 years (AAP, 2000). In children, as in adults, the majority of fire-related deaths are attributed to smoke inhalation rather than burns (AAP, 2000). Children were among the smoke-inhalation fatalities in the widely publicized apartment fires in the Paris, France area during 2005 (CNN International, 2006). In one apartment fire in August 2005, 14 of 17 fatalities were of children. In a second apartment fire also in August 2005, 4 of the 7 fatalities were of children. Children also died in a third apartment fire in September 2005.

Cyanide is an important contributor to death by smoke inhalation and is present in the blood of fire victims (regardless of age) in most cases. In a meta-analysis of smoke-inhalation associated deaths occurring in 7 major fire incidents from 1971 to 1990, cyanide was found in the victims' blood in each study in which it was measured (Alarie, 2002). Carboxyhemoglobin levels correlated poorly with blood concentrations of carbon monoxide. The percentage of fatalities having lethal blood concentrations of cyanide ranged from 33% to 87% in the meta-

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analysis. In one fire scene, for example, toxic blood concentrations of cyanide were documented in 87% of victims, although only 72% had a carboxyhemoglobin level exceeding 30%, a finding suggested by incomplete data from other scenes as well and suggesting a cause of death other than carbon monoxide in these victims. Consistent with the results of this meta-analysis, other studies have found cyanide in the blood of 62% to 77% of victims who died (Barillo et, al., 1994).

Elevated blood cyanide concentrations have been found in children exposed to fire smoke. In a seminal study of the role of cyanide in smoke-inhalation injury and death, 30 of the 100 victims of smoke inhalation in residential fires in Paris were younger than 14 years (Baud et al., 1991). Among those 30 children, 13 died and 17 survived. Cyanide was present in both children who survived (mean concentration: 27.4 nmol/L) and those who died (mean concentration: 87.0 µmol/L). Blood carbon monoxide concentrations were below the lethal level in some children who survived and some who died, a result suggesting, when considered in conjunction with the presence of cyanide in their blood that cyanide poisoning and/or other causes of hypoxia may have contributed to their death. The general population may be exposed to cyanide from ambient air, drinking-water and food. Based on an atmospheric hydrogen cyanide concentration of 190 ng/m^3 and an average daily inhalation of 20 m^3 air, the inhalation exposure of the general US non-urban, non-smoking population to hydrogen cyanide is estimated to be 3.8 µg/day (ATSDR, 1997) while based on a daily drinking-water consumption of 2 litres for an adult, the daily intake of cyanogen chloride is estimated to be 0.9–1.6 µg (equivalent to 0.4–0.7 µg of cyanide) (ATSDR, 1997) for cyanogen chloride concentrations in water of 0.45–0.80 µg/litre (0.19–0.34 µg cyanide/litre). Among the general population, subgroups with the highest potential for exposure to cyanide include active and passive smokers, individuals involved in large-scale processing of foods high in cyanogenic glycosides, individuals consuming foods high in cyanogenic glycosides, and, to a lesser degree, fire-related smoke inhalation victims.

2.7 TREATMENT OF POISONING AND ANTIDOTES

Cyanide produces a rapid onset of toxicity, which must have vigorous and immediate treatment to prevent the toxic syndrome. To obtain better protection, a series of newer antidotes either alone or in adjunction with the conventional treatments have been examined (Way et al., 1984) (Isom et al., 1985). Their mechanism of action, efficacy and toxicity have

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been reviewed as part of a joint IPCS (UNEP, ILO, WHO)/CEC project to evaluate antidotes used in the treatment of cyanide poisoning (Vanhleijst et. al., 1990). A wide variety of compounds have been used as cyanide antidotes and they have been classified into four major groups based on their mechanism of action: Scavengers, Detoxification, Physiological and Biochemical (Isom, 1995).

2.7.1 Scavengers

These are compounds that inactivate cyanide by binding it or by forming methaemoglobin, which in turn sequesters cyanide.

a. Methemoglobin formers:

The basic aim of rapid detoxification of cyanide is prevention or reversal of inhibition of cytochrome oxidase by cyanide. This is usually accomplished by providing a large pool of ferric iron in the form of methemoglobin to complex cyanide. Cyanide preferentially competes with the Fe^{+++} of methemoglobin as compared to that of cytochrome oxidase, and eventually binds with the former to form cyanmethemoglobin (Jandorf, 1946). Thereby, the activity of inhibited cytochrome oxidase is restored. The various methemoglobin formers employed as cyanide antidotes include:

(i) Amyl nitrite:

Inhalation of amyl nitrite as a first aid measure to cyanide poisoning is known for many years (Vanhleijst, 1987). However, the efficacy of amyl nitrite as methemoglobin inducer remained disputed on account of its inability to generate methemoglobin greater than 6% (Bastian, 1959), while about 15% is required to challenge one LD50 dose of cyanide (Vanhleijst, 1987). Now the protective effect of amyl nitrite is attributed to its vasodilatory effect that can reverse the early cyanide induced vasoconstriction. Artificial ventilation with amyl nitrite broken into ambu bags has been reported as a life saving therapy in cyanide poisoned dogs, prior to induction of significant level of methemoglobinemia (Vick et. al. 1985).

(ii) Sodium nitrite:

Sodium nitrite (SN) is the most prevalent drug of choice for cyanide poisoning (Chen et. al. 1952). When given intravenously (i.v.) it takes about 12 min to generate approximately 1% of methemoglobin (Vanhleijst, 1987). Inspite of this delay in inducing a significant level of methemoglobinemia, a reasonable protection offered by SN can be ascribed to its vasodilatory

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property (Vanlleijst, 1990). A serious drawback with SN is that (intra venous) its administration may be accompanied by serious cardiovascular embarrassment, particularly in children, for whom an adjusted dose is recommended (Berlin, 1977). Since SN induced methemoglobinemia impairs oxygen transport, it cannot be recommended for fire victims where in most instances HCN exposure is accompanied by carbon monoxide poisoning (Health Canada, 2002). Since carbon monoxide also impairs oxygen carrying capacity of blood, administration of SN would further aggravate the hypoxic condition. SN is also not advised for individuals with glucose- 6-phosphate dehydrogenase (G6PD) deficient red cells because of possibility of serious hemolytic reactions (Vanlleijst, 1990).

(iii) 4 - Dimethylaminophenol:

The relatively slow rate of methemoglobin formation by SN prompted the development of rapid methemoglobin formers like aminophenols. 4-dimethylaminophenol (DMAP) is the treatment of choice for cyanide poisoning in Germany. A dose of 3.25 mg/kg i.v. of DMAP was reported to produce methemoglobin level of 30% within 10 min and 15% methemoglobinemia was attained within one minute without any immediate effect on cardiovascular system. However, there are differences in individual susceptibility to DMAP, which may result in an undesirable level of methemoglobinemia even after normal therapeutic doses (Vanlleijst, 1987). Intramuscular injection of DMAP results in local abscess and fever. Its clinical application remains limited on account of its other toxicological implications like nephrotoxicity (Weger, 1983). Co-administration of a reduced dose of rapid methemoglobin inducer like DMAP and a slow inducer like SN were also found to be an effective pre-treatment against acute cyanide poisoning. This regimen by virtue of a protracted optimal level of methemoglobinemia provided sustained prophylaxis in rats (Bhattacharya et. al., 1991).

(iv) Other methemoglobin formers:

Hydroxylamine (HA) was yet another rapid methemoglobin inducer (Krusyna et. al., 1982) that was endowed with an anticonvulsive property (Wood et. al., 1975). In view of cyanide induced convulsions and the toxicity of DMAP, the efficacy of HA co administration with SN was also examined in rats (Bhattacharya et. al., 1993). Although, this regimen minimized the cyanide induced convulsions, it was less effective as compared to SN+DMAP treatment. In addition to prophylaxis, co administration of SN and DMAP or HA were also effective therapeutically (Bhattacharya, 1995), but their extrapolation to humans warranted caution in

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view of the persistent toxicity of these regimens (Bhattacharya and Sugendran, 1992) The cardiovascular implications and poor pharmacokinetics of SN led to evaluation of yet another group of methaemoglobin formers viz. aminophenones and derivatives p-aminopropiophenone (PAPP), p - aminooctanoylphenone (PAOP), p -nitrosopropiophenone (PNPP) and p -hydroxy aminopropiophenone (PHAPP)]. Out of all these agents PAPP was the most effective as prophylaxis (Marrs and Bright, 1986). Another alternative treatment of cyanide poisoning, involving stroma free methemoglobin solution (SFMS) was proposed by Ten Eyck et al (Ten Eyck et al., 1985). Intravenous administration of this solution did not impair the oxygen carrying capacity of blood as caused by most other methemoglobin formers and directly sequestered cyanide to protect a 4 X LD_{50} dose of sodium cyanide in rats. Efficacy and safety of this antidote remains to be determined in larger animals.

b. Cobalt containing compounds:

Cobalt ion which forms a stable metal complex with cyanide is an effective therapeutic agent against cyanide poisoning (Linnell, 1987). Various cobalt containing compounds known to antagonise cyanide poisoning include:

(i) Dicobalt edetate (Kelocyanor):

This agent (300 mg of dicobalt edetate in glucose solution i.v.) is the current treatment of choice in France and United Kingdom. Serious side effects like vomiting, urticaria, anaphylactoid shock, hypotension and ventricular arrhythmias have been reported in patients receiving Kelocyanor (Vanhejst, 1990).

(ii) Hydroxocobalamin (Vitamin B 12a):

This agent is perhaps the most promising cyanide antidote used in human toxicology (Vanhejst, 1987). With the exchange of hydroxyl group of hydroxocobalamin for cyanide, non-toxic cyanocobalamin (Vitamin B12) is formed. However, use of this antidote remained limited on account of the large dose required to challenge cyanide poisoning (Chisholm et al 1967). An injectable solution of hydroxocobalamin (5 g in water) is now available in France and Germany. In France a 4g hydroxocobalamin solution in 80 ml of sodium thiosulphate (STS) has also been developed. Reported side effects of hydroxocobalamin includes anaphylactoid reactions and acne

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(iii) Other cobalt compounds:

Cobaltous chloride, cobaltous acetate, cobalt histidine and sodium cobalt nitrite are also reported to antagonise cyanide poisoning. However, none of them has been used clinically (Linnell, 1987).

c. Cyanohydrin Formers:

Cyanide is a nucleophile known to react with various carbonyl moieties like ketones and aldehydes to yield cyanohydrin derivatives (Way, 1984). Sodium pyruvate was reported to effectively challenge acute cyanide poisoning in mice (Schwartz et al., 1979). Another α ketocarboxylic acid like α - ketoglutaric acid (α -KG) is currently being pursued widely as a cyanide antidote (Dulaney et al., 1991). Protective effect of α -KG was also observed against cyanide induced convulsions in mice (Yumamoto, 1990). α -KG either alone or in combination with SN and/or STS attenuated toxicity in mice exposed to cyanide through different routes (Bhattacharya and Vijayaraghavan, 1991). Prophylactic or therapeutic ability of α -KG was also shown to be augmented by oxygen (Delhumeau et al., 1994). Cyanide induced histotoxic hypoxia was reversed by α -KG which was found to be more effective than cobalt edetate and sodium pyruvate. Although, clinical trials of this agent as cyanide antidote has not yet been conducted in humans based on the promising results in experimental animals it is presently envisaged as a potential antidote for cyanide poisoning. It is considered safe as oral form of α KG is sold as an over-the counter nutritional supplement (Klaire Laboratories, San Marcos, CA) (Dulaney et al., 1991).

2.8 CYANOGENIC GLYCOSIDES

Cyanogenic glycosides are phytotoxins, which occur in at least 2000 plant species of which a number of species are used as food in some areas of the world. Cassava and sorghum are especially important staple foods containing cyanogenic glycosides (Conn, 1979; Nartey, 1980; Oke, 1980 and Rosling, 1994).

There are approximately 25 cyanogenic glycosides known. The potential toxicity of a cyanogenic plant depends primarily on:

(i) If the plant is consumed raw, or insufficiently processed, HCN may be released in the body until the low pH of the stomach deactivates β glucosidase enzyme.

(ii) The plant may not be sufficiently detoxified during processing or preparation and therefore, HCN may remain in the food.

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Several factors are important in this toxicity. The first aspect is the processing of plant products containing cyanogenic glycosides. When the edible parts of the plants are macerated, the catabolic intracellular enzyme ß-glucosidase can be released, coming into contact with the glycosides. This enzyme hydrolyzes the cyanogenic glycosides to produce hydrogen cyanide and glucose and ketones or benzaldehyde.

The hydrogen cyanide is the major toxic compound causing the toxic effects. Plant products (notably cassava), if not adequately detoxified during the processing or preparation of the food, are toxic because of the release of this preformed hydrogen cyanide.

The second aspect is the direct consumption of the cyanogenic plant. Maceration of edible parts of the plants as they are eaten can release ß-glucosidase. The ß-glucosidase is then active until the low pH in the stomach deactivates the enzyme. Additionally, it is possible that part of the enzyme fraction can become reactivated in the alkaline environment of the gut. At least part of the potential hydrogen cyanide is released, and may be responsible for all or part of the toxic effect of cyanogenic glycosides in the cases of some foods. In humans, cyanide is detoxified by the enzyme rhodanese which can further convert majority of the cyanide to a less toxic thiocyanate which is excreted in urine.

2.9 LEAFY VEGETABLES

Several vegetable species abound in Nigeria and most West African countries where they are used partly as condiments or spices in human diets or as supplementary feeds to livestock such as rabbits, poultry and swine (Aletor and Adeogun, 1995). Leafy vegetables are important items of diet in many Nigerian homes. These vegetables are harvested at all stages of growth and fed either as processed, semi-processed or fresh to man while they are usually offered fresh to livestock. The nutritional interest in some of these vegetable species stems from their rich contents of essential amino acids, vitamins and minerals. Further to their rich content of the mentioned nutrients, it is established that green vegetable leaves are the cheapest and most abundant source of proteins because of their ability to synthesize amino acids from a wide range of virtually available primary materials such as water, carbon dioxide and atmospheric nitrogen (as in legumes) (Fasuyi and Aletor, 2005).

Apart from the variety which they add to the menu, they are available sources of nutrients especially in rural areas where they contribute substantially to protein, fiber and other nutrients which are usually in short supply in daily diets (Aletor, 1990). They add flavor,

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variety, taste, color and aesthetic appeal to what will otherwise be a monotonous diet. They are in abundance shortly after the rainy seasons but become scarce during which cultivated types are used. Leafy vegetables are among the easiest to obtain and grow in the tropics. They are good sources of dietary fiber, protein, vitamins A, C, and B-complex, minerals, especially calcium, iron, magnesium, and phosphorus, and are low in carbohydrates and fats. Dark green leaves are usually more nutritious than lighter or yellowish leaves. Many leafy vegetables are perennials and yield useful food with a minimum amount of labour.

2.9.1 TELFAIRIA OCCIDENTALIS

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. Common names for the plant include fluted gourd, fluted pumpkin, iroko, and ugwu. The plant is dioecious, perennial, and drought-tolerant. It is usually grown trellised in Nigeria. *Telfairia* of different species are also grown as leaf vegetables in other tropical regions of the world including India, Bangladesh, Sri Lanka and the Caribbean. It is also grown to some extent in South East Africa and Latin America. *Telfairia* is classified in the tribe *Joliffieae* of the family *cucurbitaceae* and commonly referred to as fluted pumpkin. *Telfairia* species have been found to be rich in protein (21 - 37% CP), ash (1.8%), fat (13%) and fibre (13%) (Akoroda 1990). *Telfairia* species are also known to be rich sources of iron and essential fatty acids making it desirable as cooking oil. The essential amino acids contents compare favorably with those of important legumes (Asiegbu, 1987) and the high content of mineral and vitamin nutrients especially Fe, Mg and K, carotene and vitamin C is remarkable making the leaves potentially useful as food supplements. Another economic and nutritional advantage of *Telfairia* plant is its clear agronomic superiority over many plant protein sources. The large (up to 5 cm), dark red seed is rich in fat and protein, and can be eaten whole, ground into powder for another kind of soup. Many leafy vegetables are perennials and yield useful food with a minimum amount of labor. Leaf vegetables respond favorably to fertile growing conditions high in nitrogen. Fluted pumpkin (*Telfairia occidentalis* Hook) belongs to the family *cucurbitaceae* and it is crop of commercial importance grown across the low land humid tropics of West Africa (Nigeria, Ghana and Sierra Leone) being the major producers (Nkang et al 2003), however, there is no identifiable information on the crop in terms of varieties (FAO 1992). It is a tropical vine grown mainly for the leaves which constitute an important component of the diet of many people in West African countries (Gill 1988, Fagbemi et al 2005) and for its edible seed.

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The young shoots and leaves of the plant are the main parts used in soup. The plant is dioecious, perennial and drought tolerant. It is usually grown trellised. It needs a well-drained soil, some water and some sun. The vines will climb up to 1.5 meter. The flowers are white and dark purple. The sex of fluted pumpkin is difficult to know until after flowering which takes about 4 months after planting. This is a major constraint to its production. The female leaves are preferred by the housewives and are therefore in higher demand (Ajibade et al 2006). The green leaves of fluted pumpkins generally called "ugwu" are well known in Southern Nigeria because of their pleasant taste. The leaves are rich source of protein, oil vitamins and minerals that enhances, nourish, protect and heal the body. The green leaves are low in crude liber, rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K and also have medicinal value (Ladeji et al 1995; Ajibade et al 2006). Relative to most common vegetables, its protein content is high (Okoli and Algbeogu 1983; Ladeji et al 1995). The leaves and shoot are consumed as food. The plant also contain considerable amount of anti-nutrients such as phytic acid, tannin and saponin which could also have some hazardous health effects on its consumers (Ladeji et al 1995, Ajibade et al 2006). Due to the richness of the leaves in iron it is used to cure anaemia (Ajibade et al 2006). The seeds are also rich in oil storage reserves however at present it has very low commercial value as an oilseed, but it is potentially valuable as a high protein oilseed for human and animal food (Giami et al 1999, Nkang et al 2003). The oily seeds have lactating properties and are widely consumed by the nursing mothers (Ajibade et al 2006).

2.9.2 Scientific Classification of *Telfairia occidentalis*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Telfairia

Specie: *T. occidentalis*

Source: Alycioja, 2006

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Telfairia occidentalis is rich in sulphur containing amino acid methionine and cysteine and it is a blood purifier (Aiyeloja 2006). The moisture, crude protein, lipid, crude fiber of the vegetable is 91.9%, 1.8%, 0.8% and 1.1% respectively while the K, Na, Ca, Mg, Zn, Fe, P and Vitamin C is 2.8, 3.5, 0.5, 3.6, 1.3, 8.0, 0.4 and 160.2 (mg/100g) respectively (Eboh, 2000)

2.9.3 Amino Acid Profile of *Telfairia occidentalis*

Amino acid profile (g/16 g N) of vegetable leaf meals

Amino acids	*Telfairia occidentalis*
Alanine	6.51
Aspartic acid	6.21
Arginine	5.02
Glycine	6.10
Glutamic acid	11.01
Histidine	1.38
Isoleucine	5.10
Lysine	2.10
Methionine	2.48
Cysteine	1.08
Meth. + Cys.	4.56
Leucine	7.58
Serine	3.91
Threonine	3.81
Phenylalanine	4.85
Valine	6.20
Tyrosine	5.62
Tryptophan	3.12

Fasuyi, 2006

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2.9.4 CORCHORUS OLITORUS

Corchorus olitorius or jute is native to Africa where it is widely cultivated in both wet regions of the Sub-Sahara and drier areas of North Africa. In Southwest Nigeria, its nutritive young leaves are cooked into paste and eaten with starchy staples (Akoroda, 1988). There are between 40 and 100 species primarily in tropical regions The plant thrives in sunny spots on soils rich in organic matter and with abundant moisture. Young leaves and shoot tips can be eaten raw or cooked and contain high levels of protein and vitamin C. Leaves are shredded and made into a paste. Jute leaves can also be dried, ground into powder and stored for use during the dry season. It is grown as an annual, though it may act as a perennial in some locations. It can be planted at the beginning of the rainy season and will withstand the hot, humid months. It can also withstand some drought conditions and extremes in soil. The K, Na, Ca, Mg, Zn, Fe, P and Vitamin C is 1.2% 0.4%, 0.2%, 0.3%, 0.4%, 0.9%, 0.4% and 205.4 (mg/100g) respectively (Eboh, 2000). The fibers can be used in twine, cloth and burlap.

Leaves contain oxidase and chlorogenic acid. The folic acid content is substantially higher than that of other folacin-rich vegetables, ca 800 micrograms per 100 g (ca 75% moisture) or ca 3200 micrograms on a zero moisture basis (Chen and Saad, 1981). The seeds contain 11.3-14.8% oil (Wall and Breyer-Brandwijk, 1962), estrogenic (Sharaf et al, 1979), which contains 16.9% palmitic-, 3.7% stearic-, 1.8% behenic-, 1.1% lignoceric-, 9.1% oleic-, 62.5% linoleic-, and 0.9% linolenic- acids as well as large portions of B, Mn, Mo, and Zn. Most of the sulphur in herbage is contained in methionine and cystine within proteins. Earlier studies showed higher production of hydrogen sulphide from cyst (e)ine than from iso-S quantities of methionine or inorganic sulphate (Bird, 1972).

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2.9.5 Scientific Classification of *Corchorus olitorius*

Domain:	Eukaryota
Kingdom:	Plantae
Subkingdom:	Viridaeplantae
Phylum:	Tracheophyta
Subphylum:	Spermatophytina
Infraphylum:	Angiospermae
Class:	Magnoliopsida
Subclass:	Dilleniidae
Superorder:	Malvanae
Order:	Malvales
Family:	Tiliaceae
Subfamily:	Tilioideae
Tribe:	Corchoreae
Genus:	Corchorus
Specific epithet:	*olitorius* L.

Akeroda, 1988

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Table 2.2: Nutrical content of *Corchorus olitorus*

Corchorus olitorus leaves/ 100g is reported to contain:

Calories	43- 58
Water (H_2O)	80.4-84.1 g
Protein	4.5-5.6 g
Fat	0.3 g
Total Carbohydrate	7.6-12.4 g
Fiber	1.7-2.0 g
Ash	2.4 g
Ca	266-366 mg
P	97-122 mg
Fe	7.2-7.7 mg
Na	12 mg
K	444 mg
Beta-carotene equivalent	6,410-7,850µg
Thiamine	0.13-0.15 mg
Riboflavin	0.26- 0.53 mg
Niacin	1.1-1.2 mg
Ascorbic acid	53-80 mg

Chen and Saad. 1981

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CHAPTER THREE

MATERIALS AND METHOD

Plate 3.1: A group of five (5) rats.

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This was an experimental study in which an animal model (rat) was used to investigate the toxicity of cyanide and the intervention properties of two vegetables on cyanide toxicity

3.1 PURCHASE OF VEGETABLES

The vegetables were purchased from an agricultural farm in Ofodo, Ibadan. The vegetables were cultivated without fertilizer. They were later identified and authenticated by botanists at Botany Department University of Ibadan.

3.2 PROCEDURE FOR AQEOUS EXTRACTION OF THE TWO VEGETABLES (*CORCHORUS OLITORUS* AND *TELFAIRIA OCCIDENTALIS*)

PROCEDURE

The vegetable leaves were picked and the wet weight (100g) was taken after which 200mls of distilled water was used to blend it. After blending, the paste was poured in the cloth mesh and squeezed thoroughly to bring out the extract. The volume of the extract was taken using a measuring cylinder. This was done for the two vegetables respectively. Gloves were worn through out the procedure, to prevent contamination. The extracts were then taken to the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for lyophilization.

3.2.1 AQEOUS EXTRACTION OF 'UGWU' *TELFAIRIA OCCIDENTALIS*

Wet Weight of 'Ugwu' leaves = 100g

Volume of distilled water used = 200mls

Volume after aqueous extraction = 470mls

Weight of lyophilizing tray = 222.64g

Dry weight of 'Ugwu' extract = (Total weight of tray and extract – weight of tray) g

= (229.345 – 222.64) g

= 6.705g

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3.2.2 AQEOUS EXTRACTION OF 'EWEDU' *CORCHORUS OLITORUS*

Wet Weight of 'Ewedu' leaves = 100g

Volume of distilled water used = 200mls

Volume after aqueous extraction = 650mls

Weight of lyophilizing tray = 222.64g

Dry weight of 'Ewedu' extract = (Total weight of tray and extract – weight of trays) g

= (236.227 – 222.64) g

= 13.587g

3.3 PROCEDURE FOR LYOPHILIZATION (FREEZE DRYING)

200 mls of each vegetable extract was dispensed in trays and was frozen for 5 hours after which it was placed in the batch lyophilizer and freeze dried for 2 days. The dry weights of the vegetable extracts were taken.

3.3.1 PRINCIPLE OF LYOPHYLIZATION (FREEZE DRYING)

Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. There are however many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books and other items damaged by water and the concentration and recovery of reaction products. Freeze drying involves the removal of water and other solvents from a frozen product by a process called sublimation. Sublimation occurs where a frozen solid goes directly from solid state to the gaseous state without passing through the liquid phase (Mellor, 1978). In contrast, drying at ambient temperatures from the liquid phase usually results in changes in the product and may be suitable only for some materials. However, in freeze drying, the material does not go through the liquid phase and it allows the preparation of a stable product that is easy to use and aesthetic in appearance. The freeze drying process consist of three stages i.e. the freezing, primary drying and secondary drying.

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Pre freezing

Since freezing is a change in state from the gaseous or liquid phase to the solid phase, materials to be freeze dried must first be adequately pre frozen. The method of pre freezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material. Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry. Slower cooling results in larger ice crystals and less restrictive channels in the matrix during the drying process. Products freeze in two ways depending on the make up of the product. The majority of the product that is subjected to freeze drying consists primarily of water. Most samples that are to be freeze dried are eutectics which are a mixture of substances that freeze at lower temperatures than the surrounding water. When the aqueous suspension is cooled, changes occur in the solute concentrations of the product matrix. As cooling proceeds, the water is separated from the solutes as it changes to ice, creating more concentrated areas of solute. This pocket of concentrated materials, have a lower freezing temperature than the water. Although a product may appear to be frozen because of all the ice present, in actuality, it is not completely frozen until all of the solute in the suspension is frozen. The mixture of various concentrations of solutes, with the solvent constitutes the eutectics of the suspension. Only when all the eutectic mixture is frozen is the suspension properly frozen. This is called the eutectic temperature (Mellor, 1978). It is very important in freeze drying to pre freeze the product to below the eutectic temperature before beginning the freeze drying process. Small pocket of unfrozen material remaining in the product expand and comprise the structural stability of the freeze dried product.

Primary Drying

After pre freezing the product, conditions must be established in which ice can be removed from the frozen product via sublimation, resulting in a dry, structurally intact product. This requires very careful control of the two parameters, temperature and Pressure, involved in the freeze drying system. The rate of sublimation of ice from a frozen product depends on the difference in vapor pressure of the product compared to the vapor pressure of the ice collector. Molecules migrate from the higher pressure to a lower pressure. Since vapor pressure is related to temperature, it is necessary that the product temperature is warmer than the cold trap (ice collector) temperature (Mellor, 1978). It is extremely important that the temperature at which a product is freeze dried is balanced between the temperature that maintains the liveli integrity of the product and the temperature that maximizes the vapor pressure of the product

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Secondary Drying

After primary freeze drying is complete and all ice has sublimed, bound moisture is still present in the product. The product appears dry but the residual moisture content may be as high as 7-8 %. Continued drying is necessary at the warmer temperature to reduce the residual moisture content to optimum values (Mellor, 1978). This process is called Isothermal Desorption as the bound water is desorbed, from the product. Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity of the product. All other conditions such as pressure and collector temperature remain the same. Because the process is desorptive, the vacuum should be as low as possible (no elevated pressure) and the collector temperature as cold as can be attained. Secondary drying is usually carried out for approximately 1/3 to ½ the time required for primary drying.

3.4 PREPARATION OF THE STOCK AND WORKING CONCENTRATION

SOLUTION:

30 mg of KCN was dissolved in 100 mls of distilled water and kept in a refrigerator.

30 mg of each vegetable extract was also dissolved in 100 mls of distilled water to make a 1:10 dilution to give 30mg/kg before it was kept in a refrigerator.

WORKING CONCENTRATION:

1 mls of the KCN stock was dispensed in 9mls of distilled water, shaken and covered with a foil paper and kept in the refrigerator. This is a 1 in 10 dilution (1:10).

1ml of each of the vegetable extract was dispensed in 9 mls of distilled water in a 1 in 10 dilution, shaken and covered with a foil paper before it was kept in a refrigerator.

3.5 EXPRIMENTAL ANIMALS

Thirty (30) adult albino Wistar male rats were distributed randomly into 6 groups. Five (5) experimental groups and One (1) positive control group, in respective cages (Plate 3.1).

The animals (rats), which were from the same litter, were purchased from the animal house, Department of Physiology, University of Ibadan. The rats which were obtained at 3 weeks old and transferred to the Animal House, IMRAT, Biode Building, University College Hospital to acclimatise for four weeks. They were maintained for four weeks on commercial rat pellets and water ad- libitum until a weight range of between 160g and 280g was obtained. The cages

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and water bottles were washed thoroughly while the sawdust which served as their beddings was treated with dettol and sun dried periodically. They were fed ad-libitum with commercial rat pellet and water daily. The vegetables had earlier been extracted, lyophilised and later reconstituted with distilled water to the specified concentration before it was administered.

The groups were identified for experimentation as follows:

- Group 1: - Control.
- Group 2: - Cyanide Only
- Group 3: - Cyanide and *Telfairia occidentals* Extract (3mg/kg body weight each). (CN+TO).
- Group 4: - Cyanide and *Corchorus olitorus* Extract (3mg/kg body weight each). (CN+CO).
- Group 5: - *Telfairia occidentals* extract only (3mg/kg body weight) (TO only).
- Group 6: - *Corchorus olitorus* extract only (3mg/kg body weight) (CO only).

3.6 PROCEDURE FOR ADMINISTERING KCN AND THE VEGETABLE EXTRACTS

The animals were fed using an adjustable micropipette with plastic tips and a canular. The canular were labelled to prevent cross contamination. The weight of the rats were taken daily along with other physical observations before the toxin (KCN) and the treatment (the vegetable extracts) were administered. Volume of the toxin and the extracts administered were based on the weight of the rats. The ratio of the toxin to either the extract or distilled water was 1:1. The adjustable micropipette was used to pick the extracts, KCN and distilled water and dispensed into small bottles where it is mixed thoroughly before feeding it to rats.

The animals were picked from the tail and the neck is gripped with the left hand and turned upwards with its limbs hanging up and the tail tucked between the hollow of the left hand (Plate 3.2), then a clear passage to the throat was sought before the mixture is administered. Physiological parameters i.e. agility, eye and fur colour, nose discharge and ocular and nasal lesion were checked and recorded daily before treatments.

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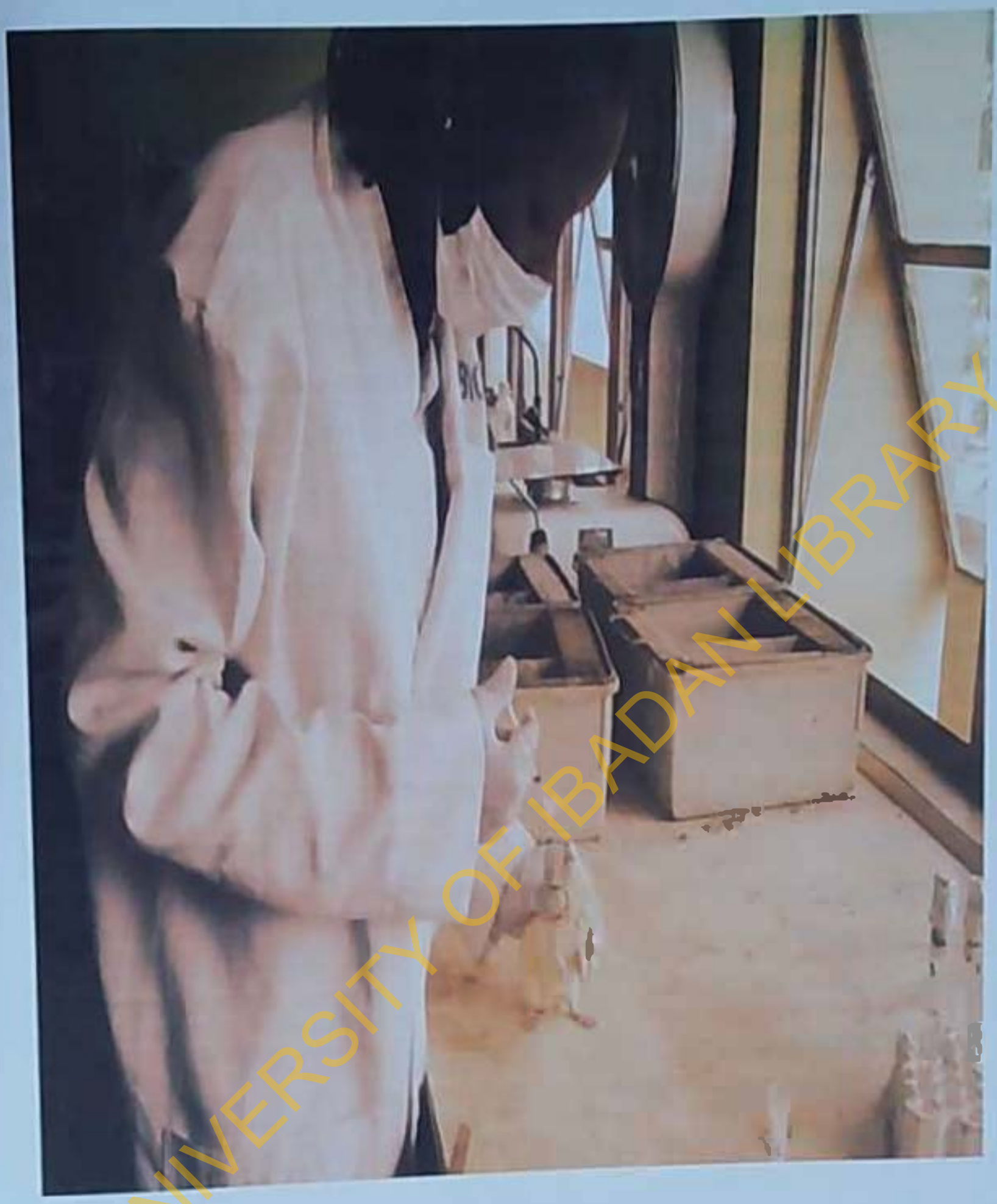

Plate 32: Researcher feeding the rats

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3.7 POST TREATMENT HARVEST

3.7.1 COLLECTION OF BLOOD SAMPLES

The rats were fasted for 24 hours before the blood samples were collected. Capillary tubes were used to collect blood samples from the rats while they were still alive using the ocular puncture method (Plate 3.3). The blood samples were placed inside Lithium heparinized bottles and were centrifuged at 1000 revolution/ minutes for 10 minutes after which the plasma was aspirated into universal bottles before they were analysed for liver function enzymes; Alkaline Phosphatase (ALP), Aspartate Amino Transferases (AST), and Alanine Amino Transferases (ALT). The Packed Cell Volume (PCV) of blood from each rat was also estimated.

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Plate 3.3: Blood collected through ocular puncture for chemical pathological analysis and PCV

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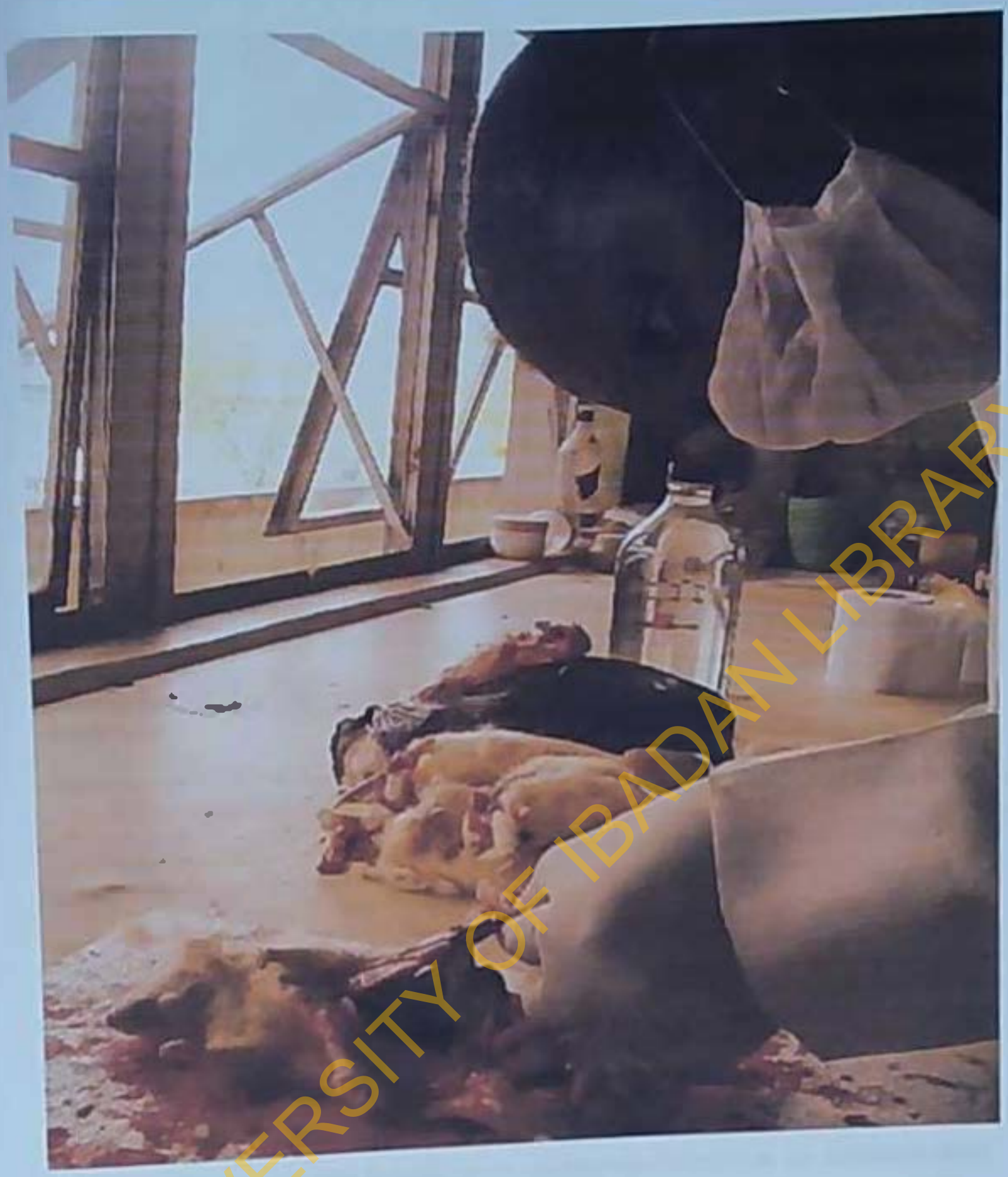

Plate 3.4: Researcher bringing out vital organs for histopathology.

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Plate 3.5: Organs collected in containers containing chloroform for histopathology

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3.7.2 Collection of Tissues

After the collection blood samples, the animals were sacrificed (Plate 3.4). This was carried out using the Cervical Dislocation method. The carcass was cut open and the kidney, liver and brain tissues were removed and placed inside 10% Formalin for histopatological analysis (Plate 3.5).

3.8 TOTAL PROTEIN ANALYSIS USING KIELDAHL METHOD

This analysis was carried out on the two vegetable extracts *Corchorus olitorius* and *Telfairia occidentalis* at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

3.8.1 Nitrogen/ Phosphorus Digestion Procedure

Apparatus

Hot plate, Pyrex conical flask, volumetric flask, oven, weighing balance, distilled water, aluminium blocks, digestion tubes, auto-analyzer.

Reagents

(Sulphuric acid) H_2SO_4, selenium powder, (Ammonium tetraoxosulphate) $(NH_4)2SO_4$, (Potassium dihydrophosphate) KH_2PO_4, H_2O_2 (Hydrogen peroxide).

Procedure

Selenium/Sulphuric acid Mixture: - 1 litre of sulfuric acid was added to 3.5 g of selenium powder. It was then heated on a hotplate at high temperature until clear. The selenium dissolved into the sulfuric acid at about 280 °C. After the selenium has dissolved, the hotplate was turned off and the container was left on the hotplate until cool.

Nitrogen/Phosphorus Stock Solution: To a 100 ml volumetric flask, 4.714 g $(NH_4)2SO_4$ and 0.439 g of KH_2PO_4 was added. The chemicals were oven-dried at 105 C before weighing and diluted to mark. This solution contains 10,000 ppm N and 1000 ppm P.

1) 0.200 g of dry, ground plant sample was weighed into a 50 ml digestion tube and 2.5 ml of the H_2SO_4/Se mixture was added to each tube and to 5 blanks which was used for standards. It was then placed in an aluminium block on a hotplate and heated at approximately 200°C until the samples started fuming.

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2) The tubes were removed from the hotplate and allowed to cool for 10 minutes. 1 ml of 30% H_2O_2 was carefully added to the samples and standards. After the reaction has subsided an additional 2ml H_2O_2 was added.

3) It was replaced on the hotplate and a heavy 15 ml glass vial was placed on top of each tube and heated to 330°C. It was left on the hot plate until clear (usually 2 hours). The yellow tint of the samples should disappear as the digest is completed.

4) The samples were allowed to cool. To the 5 standard solutions 0, 0.200, 0.400, 0.600, and 0.800 ml of the N/P stock solution was added. The samples and standards were diluted to the 50 ml mark. Each sample was placed into the auto- analyzer cups and the Nitrogen and Phosphorous was read on the auto-analyzer machine.

Precautions:

The container used to heat the sulphuric acid/selenium mixture is made of sturdy Pyrex glass. A breakage would be extremely dangerous.

3.9 DATA AND STATISTICAL ANALYSIS

Inferential statistical analysis was performed on the data collected from the experiment which includes, a daily record of body weight, food and drinking water consumption. In comparing the results of the groups, ANOVA (analysis of variance) and student 't' test was applied and the difference was taken to be significant when P- value is ≤0.05 to ascertain the significance of the intervention.

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CHAPTER FOUR

RESULTS

4.1 PROTEIN ESTIMATION RESULT FOR *Telfairia occidentalis*

The percentage of protein in *Telfairia occidentalis* were 31.96% respectively for the two samples from the same source (Table 4.1).

4.2 PROTEIN ESTIMATION RESULT FOR *Corchorus olitorus*..

The percentage of protein in *Corchorus olitorus* was 40.28% respectively for the two samples from the same source (Table 4.2).

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Table 4.1: Percentage of protein in *Telfairia occidentalis*

	% PROTEIN
SAMPLE 1	31.96
SAMPLE 1	31.96

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Table 4.2: Percentage of protein in *Corchorus olitorius*

	% PROTEIN
SAMPLE 2	40.28
SAMPLE 2	40.28

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Table 4.3: Means for average water intake in the 6 groups

Average Water Intake (ml)

GROUPS	N	Subset for alpha = .05		
		1	2	3
Control	14		24.0000	24.0000
CN ONLY	14	18.2143		
CN+TO	14	17.1000		
CN+CO	14	18.8286	18.8286	
TO ONLY	14		24.1429	24.1429
CO ONLY	14			25.5714
Prob.		.542	.059	.579

Means for groups in homogenous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 14.000

Key: CONTROL = Control
CN ONLY = Cyanide only
CN+TO = Cyanide + Ugwu extract (*Telfairia occidentalis*)
CN+CO = Cyanide + Uwedu extract (*Corchorus olitorius*)
TO ONLY = Ugwu extract only
CO ONLY = Uwedu extract only

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4.3 Interactive Graph for Average Water Intake

The average water intake for rats in groups one to six for the period of fourteen days were 24.0±1.6ml. 18.2±8.8ml. 17.1± 9.7ml, 18.8±8.9ml. 24.1±3.4ml, 25.6±2.8ml respectively $p<0.05$(Table 4.3). This indicated that cyanide reduced water intake and that combination with the extracts did not affect reduction. Extracts alone did not affect water intake in the groups (Groups 5 and 6) fed with extracts only.

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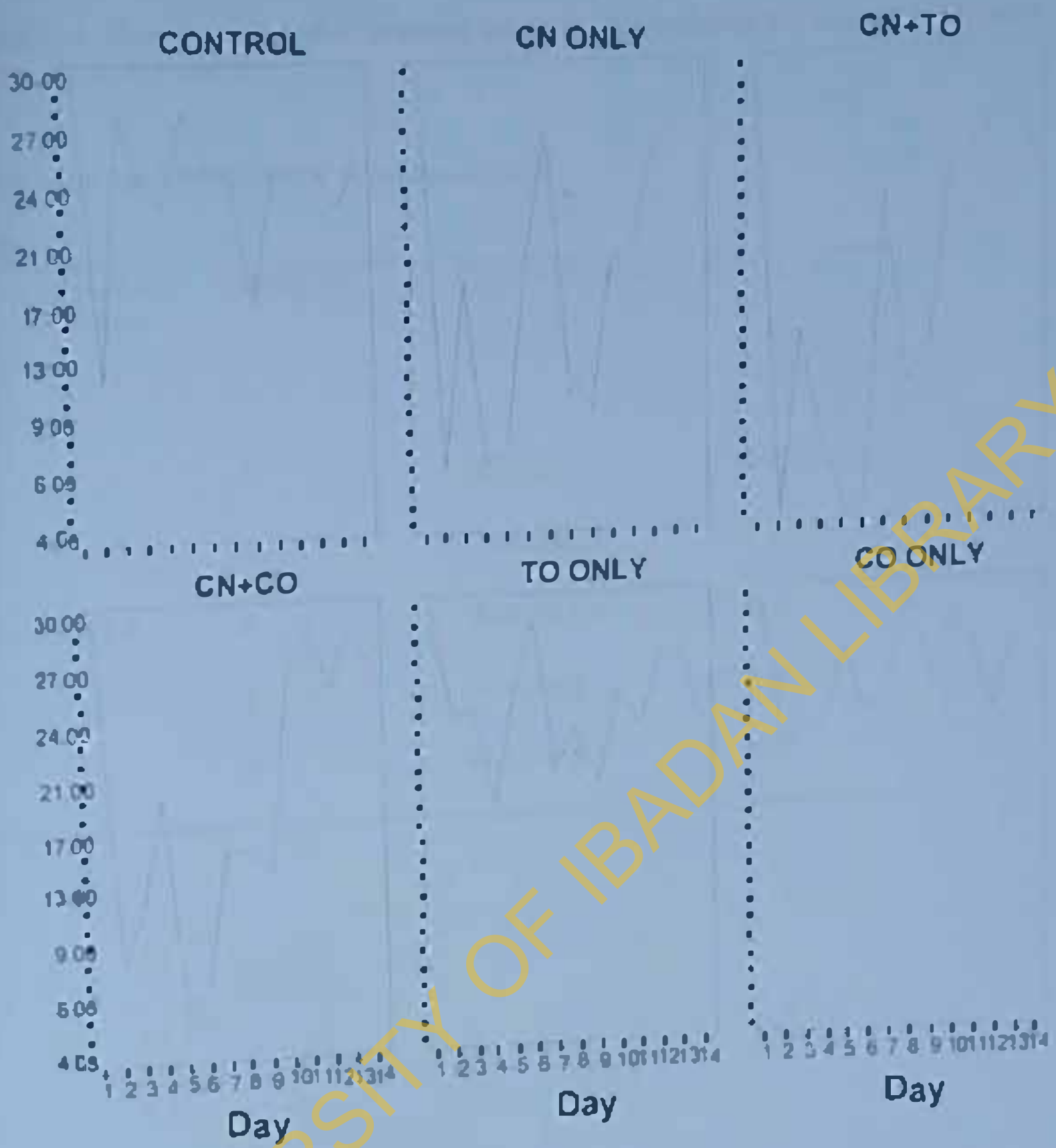

Figure 4.1: The trend in water intake for 14 days in the 6 groups

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Table 4.4: Multiple comparism between the mean water intake for control group and groups 2, 3, 4, 5 and 6.

Dependent Variable: Average Water Intake (ml)

LSD

CONTROL mean (ml)	GROUPS	TEST MEAN (ml)	P-VALUE
	2	18.2143±8.8	P< 0.05
	3	17.1000±9.7	P< 0.05
24.0000±4.6	4	18.8286±8.9	P> 0.05
	5	24.1429±3.4	P> 0.05
	6	25.571.1±2.8	P> 0.05

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Table 4.5: Means for average food intake

Average Food Intake (g)

		N	Subset for alpha = .05	
			1	2
Group 1	CONTROL	14	31.1429	
Group 2	CN ONLY	14		
Group 3	CN+TO	14	26.2143	26.2143
Group 4	CN+CO	14	26.8429	26.8429
Group 5	TO ONLY	14	26.4286	26.4286
Group 6	CO ONLY	14		30.3571
	Prob.		.108	.251

Means for groups in homogenous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 14.000

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Table 4.6: Comparism between the control group and group 2, 3, 4, 5 and 6.

Dependent Variable : Average Food Intake (g)

LSD

CONTROL mean (g)	GROUPS	TEST MEAN (ml)	P- VALUE
	2	20.1429±9.8	P< 0.05
	3	26.2143±9.8	P> 0.05
31.1429± 9.7	4	26.8429±9.6	P> 0.05
	5	26.4286±10.6	P> 0.05
	6	30.3571±10.2	P> 0.05

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4.4 FOOD INTAKE

The average food intake for rats in groups one to six are; 31.1 ± 9.7g, 20.1± 9.8g, 26.2 ± 9.8g, 26.8±9.6g, 26.4± 10.6g and 30.4± 10.2g respectively (p >0.05) (Table 4.5). In group one, there was a general increase in food intake from day one to twelve.

4.4.1 Interactive Graph for Average Food Intake

An increase in food intake was observed between day one and two, day seven to nine, day eleven to twelve and sharp reduction in food intake on days three, four, five, six, ten, thirteen and fourteen in group two. In group three, there was an increase in food intake on days one, two, four, seven, nine, ten, eleven, twelve and thirteen with sharp reduction in food intake on days three, five, eight and thirteen. There was an increase in food intake on day two, five, eight, ten, eleven and twelve while a reduction was observed on day four, six, nine and thirteen in group 4. In group 5, there was a steady increase in food intake from day two to day three with a sharp reduction on day four and an increase on day five. There was a steady increase from day six to day nine with a reduction on day ten. A reduction in food intake was observed from day eleven to day thirteen (Figure 4.2).

There was a steady increase in food intake from day one to day five with a reduction from day six to day eight and an increase from day nine to day twelve and an increase from day thirteen to fourteen in group of six.

4.5 MULTIPLE COMPARISM OF AVERAGE FOOD INTAKE BETWEEN GROUPS

Using the Fisher's Least Significant Difference (LSD) to compare Average Food Intake, the following results were obtained:

4.5.1 AVERAGE FOOD INTAKE (AFI)

4.5.2 COMPARING GROUP 1 (CONTROL) WITH GROUP 2 (CN ONLY), 3 (CN+TO), 4 (CN+CO), 5 (TO ONLY) AND 6(CO ONLY)

1. The mean differences in average food intake between group 1 and 2 was 11.0 at p < 0.05 indicating that the average food intake in the control was significantly greater than that of group 2 and that cyanide reduced appetite (Table 4.6).
2. The mean difference in average food intake between group 1 and 3 was 4.93 at p > 0.05 indicating that the AFI in the control was not significantly greater than that of

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group 3 and that *Telfairia occidentalis* annulled the reduction effects of cyanide on food intake.

3. The mean difference in AFI between group 1 and 4 was 4.30 at $p > 0.05$ indicating that there was no significant difference in food intake between group 1 and 4 and that *Corchorus olitorus* annulled the reductive effects of cyanide on food intake.
4. The mean difference in AFI between group 1 and 5 was 4.71 at $p > 0.05$ also indicating that there was no significant difference in food intake between the two groups.
5. The mean difference in AFI between Group 1 and 6 was 0.70 at $p > 0.05$ indicating that there was no significant difference in food intake between group 1and 6.

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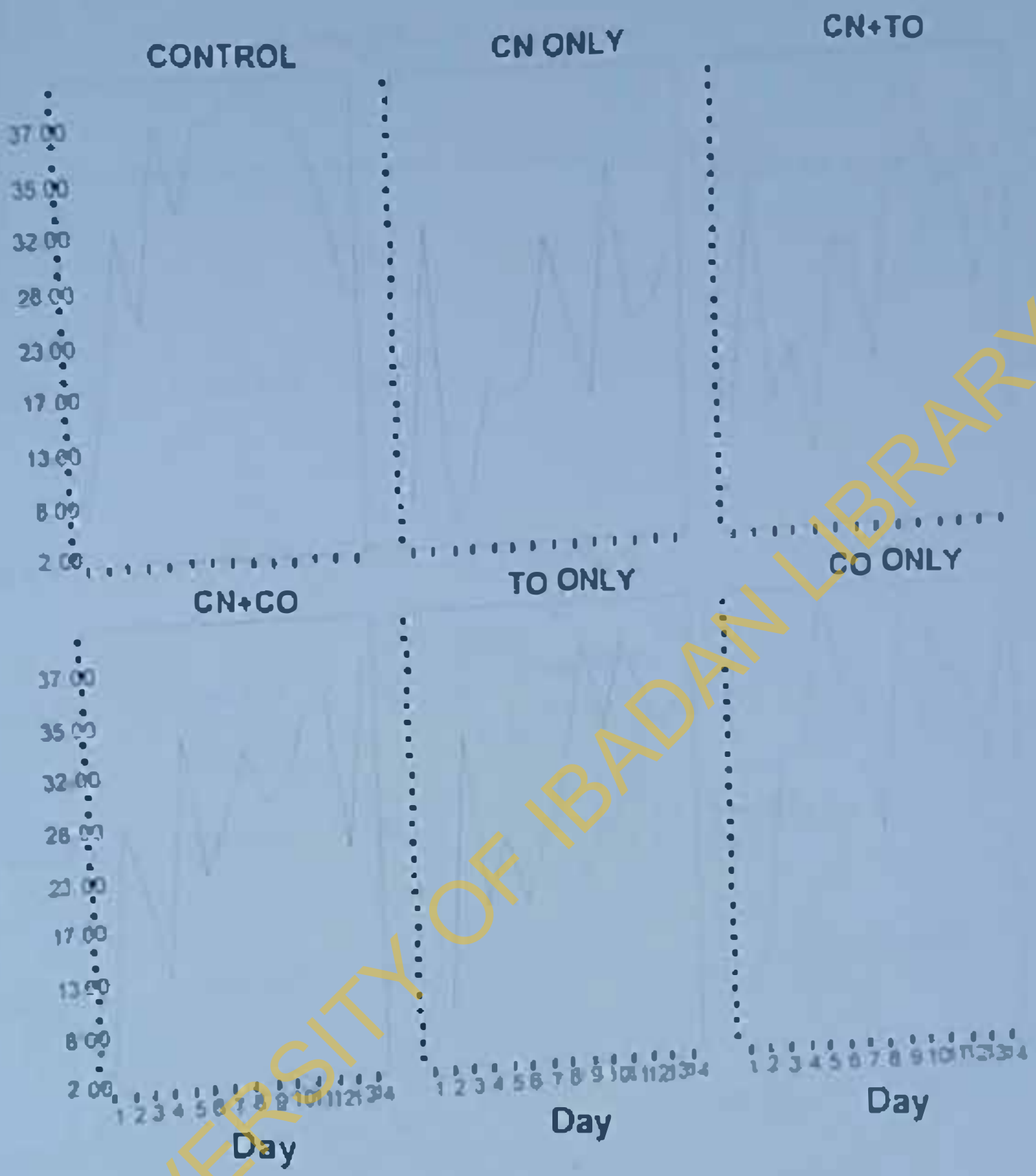

Fig 4.2: Graph showing the trend in food intake for 14days in the 6 groups.

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Table 4.7: Average body weight in groups 1 to 6

Average Body Weight (g)

		N	Subset for alpha = .05			
			1	2	3	4
GROUP 1	CONTROL	70				238.8571
GROUP 2	CN ONLY	70	188.4286			
GROUP 3	CN+TO	70	196.5714			
GROUP 4	CN+CO	70			215.7143	
GROUP 5	TO ONLY	70			231.1429	231.1429
GROUP 6	CO ONLY	70		223.2857	223.2857	
Prob.			.089	.114	.101	.107

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Table 4.8: Shows comparism between the Control and groups 2, 3, 4, 5 and 6.

Dependent Variable: Average Body Weight (g)

LSD

CONTROL mean (g)	GROUPS	TEST MEAN (g)	p. VALUE
238.8571±30.4	2	188.4286±15.9	P< 0.05
	3	196.5714±34.9	P< 0.05
	4	215.7143±19.2	P< 0.05
	5	231.1429±38.4	P> 0.05
	6	223.2857±21.2	P< 0.05

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4.6 BODY WEIGHT

The weight of the rats were taken and recorded daily after which the mean weight/ day was calculated.

The mean weights for rats in groups one to six were 238.9±30.4g, 188.4±15.9g, 196.6±34.9g, 215.7±19.2g, 231.1±38.4g and 223.3±21.2g ($p<0.05$) respectively (Table 4.7) while the average weight change for groups one to six were 24.0 ± 47.6g, -7.0± 10.7 g, 0± 33.5g, -5.0 ± 10.5g, 3.3±10.3g and 12.0±20.4g respectively thus indicating a general decline in the body weight of rats in groups two, five and six. In group 2 there was a steady reduction in body weight, (Fig 4.3).

4.6.1 AVERAGE BODY WEIGHT (ABW)

4.6.2 COMPARING GROUP 1(CONTROL) WITH GROUP 2(CN ONLY), 3(CN+ CO), 4(CN+ CO), 5 (CO ONLY) AND 6(CO ONLY).

1. The mean difference in ABW between group 1(Control) and group 2(CN only) was 50.43 at $p < 0.05$ indicating a great significant difference between group 1 and 2 and that there was loss of body weight as a result of cyanide
2. The mean difference in ABW between group 1 and group 3 was 42.29 at $p < 0.05$ indicating a significant difference in body weight between group 1 and 3 and that the reduction effect of cyanide on body weight was slightly reduced by *Telfairia occidentalis*.
3. The mean difference in ABW between group 1 and group 4 was 23.14 at $p < 0.05$ indicating a significant difference in body weight between group 1 and 4 and that the reduction effect of cyanide on body weight was significantly reduced by *Garcinia kola* (Table 4.8).
4. The mean difference in ABW between group 1 and group 5 was 7.71 at $p > 0.05$ indicating that there was no significant difference in body weight between group 1 and 5
5. The mean difference in ABW between group 1 and 6 was 15.51 at $p < 0.05$ indicating a significant difference in body weight between group 1 and 6.

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Fig 4.3: Trend in average body weight for 14 days in the 6 groups.

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Table 4.9: Percentage with and without ocular lesion in the 6 groups.

Crosstab

GROUPS		Ocular Lesion A	P	Total
CONTROL	Count	70		70
	% within Group	100.0%		100.0%
CN ONLY	Count	23	47	70
	% within Group	32.9%	67.1%	100.0%
CN+TO	Count	58	12	70
	% within Group	82.9%	17.1%	100.0%
CN+CO	Count	50	20	70
	% within Group	71.4%	28.6%	100%
TO ONLY	Count	69	1	70
	% within Group	98.6%	1.4%	100%
COONLY	Count	70		70
	% within Group	100.0%		100.0%
Total	Count	340	80	420
	% Group	81.0%	19.0%	100.0%

Key: A - Absent
P - Present

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4.7 Ocular Lesion

There was no visible sign of ocular lesion in groups one (Control) and six (CO only). In group two (CN only), 67.1% of rats have ocular lesion (Plate 4.1) while 17.1%, 28.6% and 1.4% of rats in Groups three (CN+ IO), four (CN+ CO) and five (IO only) have ocular lesion respectively $p<0.05$ (Table 4.9).

Cyanide caused ocular lesion which was almost completely reversed by treatment with *Telfairia occidentalis* and on co- treatment *Curcuma olitorius* also ameliorated the effects to a significant proportion. (Fig 4.4)

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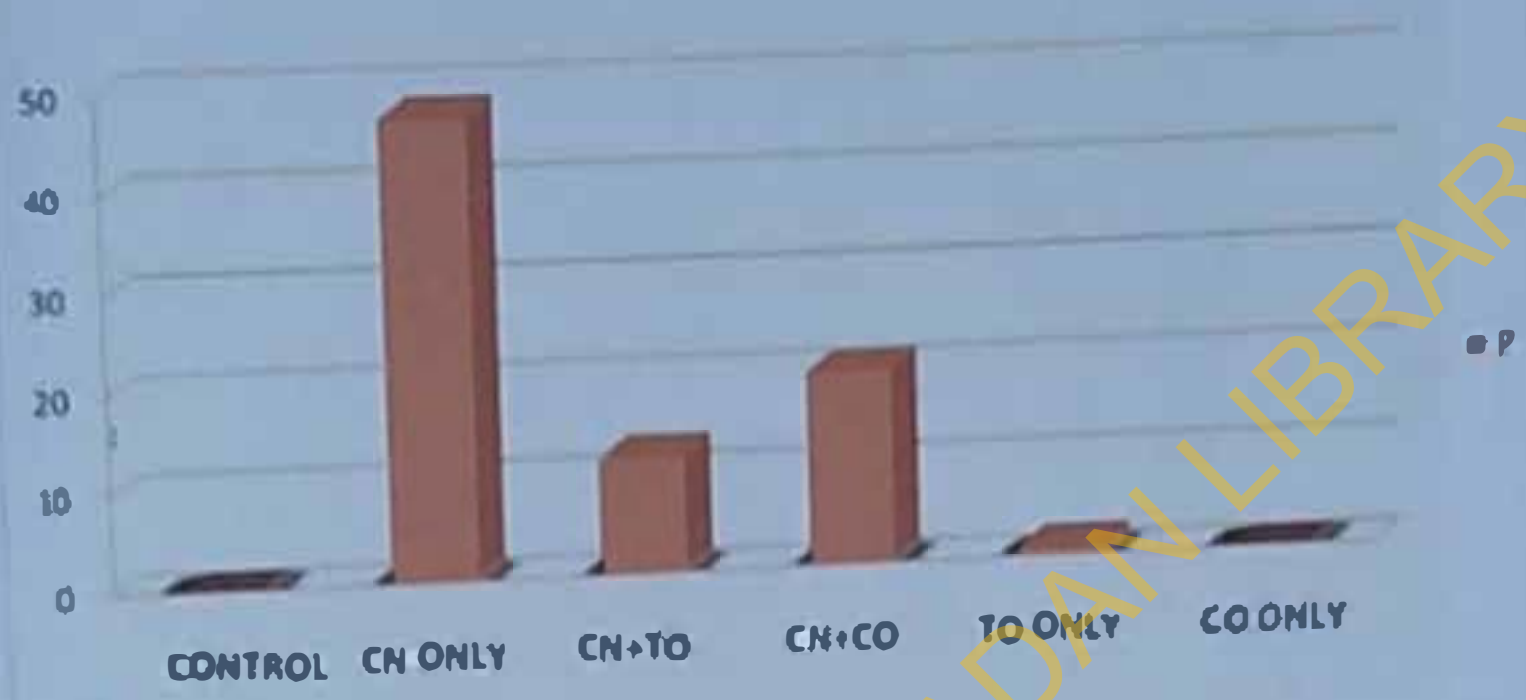

Key:
P - Present

Fig 4.4: Histogram showing the trend in ocular lesion in the six groups

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Plate 4.1: A rat with ocular lesion in Group 2 (CN ONLY)

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Table 4.10: Percentages with and without nasal lesion in the 6 groups

Crosstab

GROUPS		Nasal Lesion A	P	Total
CONTROL	Count	70		70
	% within Group	100.0%		100.0%
CN ONLY	Count	43	27	70
	% within Group	61.4%	38.6%	100.0%
CN+IO	Count	55	15	70
	% within Group	78.6%	21.4%	100.0%
CN+CO	Count	53	17	70
	% within Group	75.7%	24.3%	100%
IO ONLY	Count	70	59	70
	% within Group	100.0%	14.0%	100%
CO ONLY	Count	70		70
	% within Group	100.0%		100.0%
Total	Count	361		420
	% Group	86.0%		100.0%

KEY: A- Absent
P- Present

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4.8 Nasal Lesion

In groups one, five and six, there was no sign of nasal lesion(Plate 4.2) while in groups two, three and four 38.6%, 21.4% and 24.3% of the rats had nasal lesion respectively p<0.05 (Table 4.10) Cyanide stimulated nasal lesion which was slightly ameliorated by both *Telfairia occidentalis* and *Corchorus olitorius* on co-treatment (Fig 4.5).

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Key: P - Present

Fig 4.5: Histogram showing the trend in nasal lesion

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Plate 4.2: A rat with wound infection

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Table 4.11: Percentages with or without nasal discharge in the 6 groups

Crosstab

GROUP		Nasal Discharge		Total
		A	P	
CONTROL	Count	70		70
	% within Group	100.0%		100.0%
CN ONLY	Count	50	20	70
	% within Group	71.4%	28.6%	100.0%
CN+TO	Count	70		70
	% within Group	100.0%		100.0%
CN+CO	Count	54	16	70
	% within Group	77.1%	22.9%	100.0%
TO ONLY	Count	70		70
	% within Group	100.0%		100.0%
CO ONLY	Count	70		70
	% within Group	100%		100.0%
Total	Count	384	36	420
	% within Group	91.4%	8.6%	100.0%

KEY: A- Absent P- Present

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4.9 Nasal Discharge

No stims discharge (Fig 4.6) was found in groups one. three. five and six while 28.6% and 22.9% of rats in groups two and four had nasal discharge respectively $p<0.05$(Table 4.11) Cyanide caused nasal discharge which was completely stopped on co- treatment with *Telfairia occidentalis*. *Corchorus olitorus* also reduced the nasal discharge significantly on co-treatment with cyanide.

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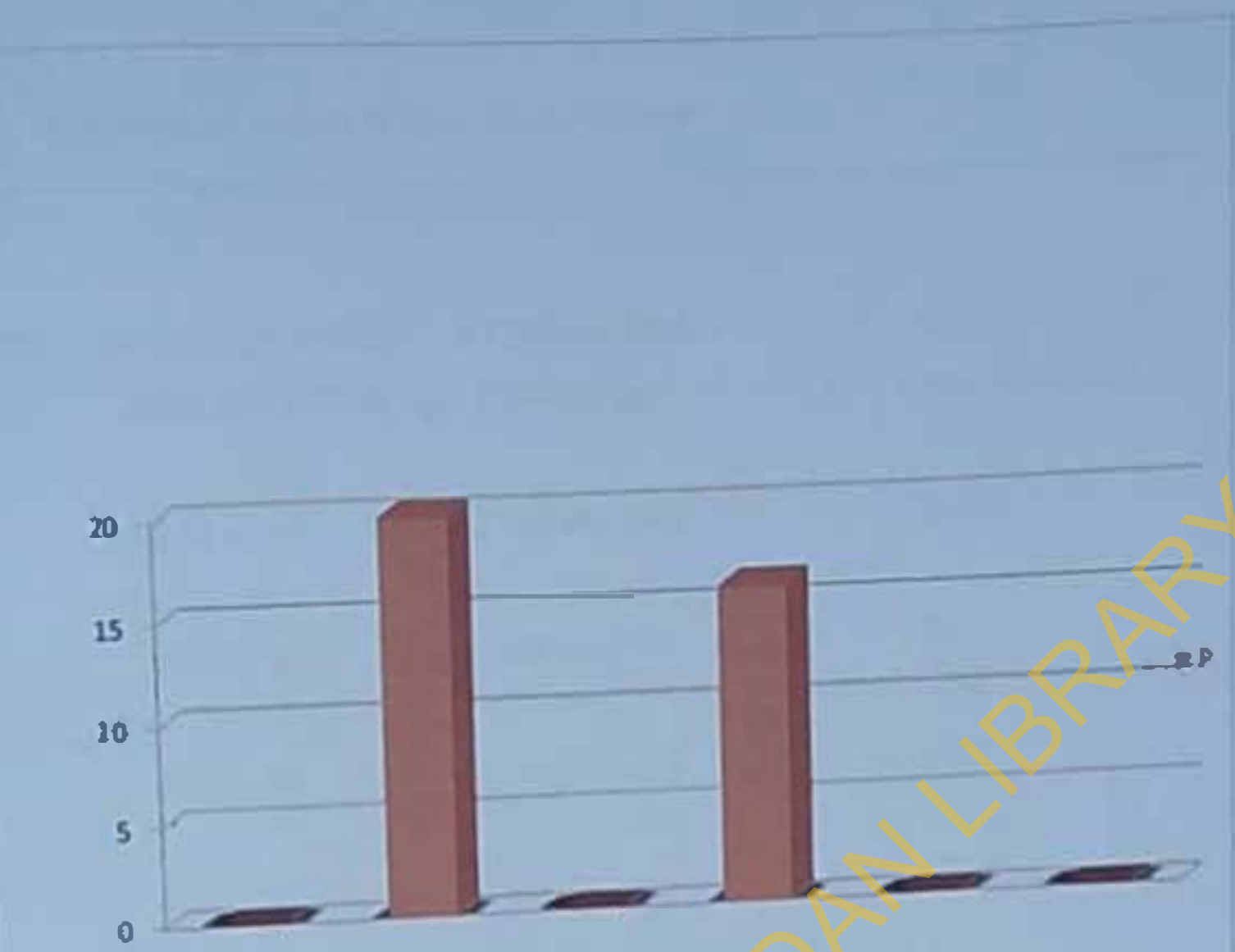

Fig 4.6: Histogram showing the trend of Nasal Discharge in the six groups

KEY:

NS. Not Slimy
S. Slimy

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Table 4.12: Shows the Organ Weight Ratio for liver

GROUPS	AVERAGE ORGAN WEIGHT (LIVER) (g)	AVERAGE BODY WEIGHT (g)	ORGAN WEIGHT RATIO(OWR)
1	4.82 ±1.01	238.86± 30.4	0.020
2	5.15±0.56	188.43±15.9	0.027
3	4.99±0.60	196.57± 34.9	0.025
4	5.12±0.56	215.71±19.2	0.023
5	4.79±0.76	231.14±38.4	0.021
6	4.68±0.62	223.29±24.2	0.021

+ ORGAN WEIGHT RATIO = $\frac{\text{AVERAGE ORGAN WEIGHT}}{\text{AVERAGE BODY WEIGHT}}$

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4.10 LIVER WEIGHTS AND CALCULATIONS FOR ORGAN WEIGHT RATIO (OWR)

1. The mean liver weight of rats in group 1 was 4.82±1.01 with an OWR (Table 4.12) of 0.020
2. The mean liver weight of rats in group 2 was 5.15 ± 0.56 with an OWR of 0.027
3. The mean liver weight of rats in group 3 was4 .99 ± 0.60 with an OWR of 0.025
4. The mean liver weight of rats in group 4 was 5.13± 0.56 with an OWR of 0.023
5. The mean liver weight of rats in group 5 was 4.79± 0.76 with an OWR of 0.021
6. The mean liver weight of rats in group 6 was 4 .68 ±0.62 with an OWR of 0.021

CN caused enlargement of the liver though with no significant difference between the groups

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Table 4.13: Average organ (kidney) weight and organ weight ratio for the 6 groups

GROUPS	AVERAGE ORGAN WEIGHT (KIDNEY) (g)	AVERAGE BODY WEIGHT (g)	ORGAN WEIGHT RATIO (OWR)
1	1.15±0.20	238.86±30.4	0.0048
2	1.27±0.15	188.43±15.9	0.0067
3	1.27±0.11	196.57±34.9	0.0064
4	1.10±0.10	215.71±19.2	0.0051
5	1.11±0.07	231.14±38.4	0.0048
6	1.17±0.14	223.29±24.2	0.0052

+ORGAN WEIGHT RATIO = $\frac{\text{AVERAGE ORGAN WEIGHT}}{\text{AVERAGE BODY WEIGHT}}$

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4.11 KIDNEY WEIGHTS AND CALCULATIONS FOR ORGAN WEIGHT RATIO

1. The mean kidney weight of rats in group 1 was 1.15 ± 0.20 (Table 4.13) with an organ weight ratio (OWR) of 0.0048.
2. The mean kidney weight of rats in group 2 was 1.27 ± 0.15 with an organ weight ratio (OWR) of 0.0067.
3. The mean kidney weight of rats in group 3 was 1.27± 0.11 with an organ weight ratio (OWR) of 0.0064.
4. The mean kidney weight of rats in group 4 was 1.10 ± 0.10 with an organ weight ratio (OWR) of 0.0051.
5. The mean kidney weight of rats in group 5 was 1.11± 0.07 (Table 4.13) with an organ weight ratio (OWR) of 0.0048.
6. The mean kidney weight of rats in group 6 was 1.17 ± 0.14 with an organ weight ratio (OWR) of 0.0052.

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Table 4.14: Show comparism between the mean kidney weight for group 1 (control) with group 2, 3, 4, 5 and 6

CONTROL Mean (g)	GROUPS	TEST MEAN (g)	P- VALUE
1.15±0.20	2	1.27±0.15	P> 0.05
	3	1.27±0.11	P> 0.05
	4	1.10 ±0.10	P> 0.05
	5	1.11±0.07	P> 0.05
	6	1.17 ±0.14	P> 0.05

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Table 4.15: Show average organ (brain) weight and organ weight ratio for the 6groups

GROUPS	AVERAGE ORGAN WEIGHT (BRAIN) (g)	AVERAGE BODY WEIGHT (g)	ORGAN WEIGHT RATIO (OWR)
1	1.64±0.13	238.86± 30.1	0.0068
2	1.73±0.08	188.43±15.9	0.0091
3	1.56±0.31	196.57±34.9	0.007a
4	1.75±0.11	215.71±19.2	0.0081
5	1.49±0.32	231.14±38.4	0.0065
6	1.58±0.29	223.29±24.2	0.0071

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4.12 BRAIN WEIGHTS AND CALCULATIONS FOR ORGAN WEIGHT RATIO

1. The mean organ (brain) weight of rats in group 1was 1.64± 0.13 with an organ weight ratio (OWR) of 0.0068 (Table 4.15).
2. The mean organ (brain) weight of rats in group 2was 1.73 ±0.08 with an organ weight ratio (OWR) of 0.0091.
3. The mean organ (brain) weight of rats in group 3was 1.56 ±0.31with an organ weight ratio (OWR) of 0.0079.
4. The mean organ (brain) weight of rats in group 4 was 1.75± 0.11with an organ weight ratio (OWR) of 0.0081.
5. The mean organ (brain) weight of rats in group 5 was 1.49 ±0.32 with an organ weight ratio (OWR) of 0.0065.
6. The mean organ (brain) weight of rats in group 6 was 1.58 ±0.29 with an organ weight ratio (OWR) of 0.0071.

4.12.1 MEAN BRAIN WEIGHT

4.12.2 COMPARING GROUP 1 (CONTROL) WITH GROUPS 2(CN ONLY), 3(CN+TO), 4(CN + CO), 5(TO ONLY) AND 6(CO ONLY).

The mean difference between the mean brain weight of rats in group 1 when compared with those of groups 2, 3, 4, 5 and 6 was -0.09, 0.08, -0.11, 0.15 and 0.06 at p> 0.05. Indicating that there was no significant difference in the mean brain weight of rats in group 1 when compared with those of groups 2, 3, 4, 5 and 6.

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Table 4.16: Show the mean concentration for ALT, ALP, AST and PCV in Group1 to 6

Mean Concentration (U/L)	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
ALT	36.4±23.75	46.4±25.69	29.8±20.4	57.6±18.36	41.8±24.53	34.8±23.82
ALP	29.6±17.42	106.2±40.05	27.6±24.54	57.0±25.87	40.4±32.73	46.2±20.85
AST	32.6±24.4	123.2±32.62	27.6±24.54	68.6±38.69	30.6±10.78	46.8±13.48
PCV	71.2±3.06	71.6±1.85	67.2±3.60	72.8±2.40	72.6±1.49	66.6±1.85

KEY: ALT = Alanine Transaminase
ALP = Alkaline Phosphatase
AST = Aspartate Transaminase

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4.13 RESULT FROM BIOCHEMICAL ANALYSIS FOR LIVER FUNCTION ENZYMES

The mean concentration for ALT for rats in groups one to six are 36.4± 23.75, 46.4± 25.69, 29.8±20.4, 57.6± 18.36, 41.8±24.53 and 34.8±23.82 respectively (Table 4.16)

The mean concentration for ALP in groups one to six are 29.6± 17.42, 106.2±40.05, 27.6± 24.54, 57.0± 25.87, 40.4± 32.73 and 46.2± 20.85 respectively (Table 4.16)

The mean concentration for AST for rats in groups one to six are 32.6±24.1, 123.2±32.62, 27.6±24.54, 68.6± 38.69, 30.6± 10.78 and 46.8±13.48 respectively (Table 4.16).

The mean concentration for PCV in groups one to six are 71.2±3.06, 70.6±1.85, 67.2±3.60, 72.8± 2.40, 72.6±1.49 and 66.6± 1.85 respectively (Table 4.16)

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Table 4.17: Show comparism between the mean ALT for group 1 with group 2, 3, 4, 5 and 6

CONTROL Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
	2	46.4± 25.69	P> 0.05
	3	29.8±20.4	P> 0.05
36.4± 23.75	4	57.6±18.36	P> 0.05
	5	41.8±24.53	P> 0.05
	6	34.8±23.82	P> 0.05

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4.13.1 ALANINE TRANSAMINASE (ALT)
MEAN ALT (mean ALT)

4.13.2 COMPARING GROUP 1 (CONTROL) WITH GROUPS 2(CN ONLY), 3(CN+TO), 4(CN + CO), 5(TO ONLY) AND 6(CO ONLY).

The mean difference in the mean ALT concentration between groups 1 and 2 was -10.0 ± 2.17 at $p < 0.05$ indicating that there was a significant difference in the mean ALT concentration between the 2groups (Table 4.17).

The mean difference in the mean ALT concentration between groups 1 and 3 was 6.6 ±6.08 at $p > 0.05$ indicating that there was no significant difference in the mean ALT concentration between the 2groups (Table 4.17).

The mean difference in the mean ALT concentration between groups 1 and 4 was – 21.2 ±6.02 at $p > 0.05$ indicating that there was no significant difference in the mean ALT concentration between the 2groups (Table 4.17).

The mean difference in the mean ALT concentration between groups 1 and 5 was -5.4 ±0.88 at $p > 0.05$ indicating that there was no significant difference in the mean ALT concentration between the 2groups.

The mean difference in the mean ALT concentration between groups 1 and 6 was 1.6 ±0.1 at $p > 0.05$ indicating that there was no significant difference in the mean ALT concentration between the 2groups.

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Table 4.18: Show comparism between the mean ALP for group 1 with group 2, 3, 4, 5 and 6

CONTROL Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
29.6± 17.42	2	106.2±40.05	P> 0.05
	3	27.6±24.54	P> 0.05
	4	57.0±25.87	P> 0.05
	5	40.4±32.73	P> 0.05
	6	46.2±20.85	P> 0.05

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Table 4.19: Show comparism between the mean ALP for group 2with group 3 and 4

GROUP 2 (CN ONLY) Mean U/L.	GROUPS	TEST MEAN U/L	MEAN DIFFERENCE	P- VALUE
106.2±40.05	3	27.6±24.54	78.6	P> 0.05
	4	57.0±25.87	49.2	P> 0.05

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Table 4.20: Shows comparism between the mean ALP for group 3 with group 5 and 6

GROUP 3 (CN+ EE) Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
27.6±24.54	5	40.4±32.73	P> 0.05
	6	46.2±20.85	P> 0.05

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Table 4.21: Show comparism between the mean ALP for group 4 with group 5 and 6

GROUP 4 (CN + UE) Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
57.0±25.87	5	40.4±32.73	P> 0.05
	6	46.2±20.85	P> 0.05

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4.1.4 ALKALINE PHOSPHATASE (ALP)

MEAN ALP (mean ALP)

4.1.4.1 COMPARING THE MEAN GROUP 1 (CONTROL) WITH GROUPS 2(CN ONLY), 3(CN+ TO), 4(CN + CO), 5(TO ONLY) AND 6(CO ONLY)

The mean difference in the mean ALP concentration between groups 1 and 2 was -76.6 ±25.3 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.18).

The mean difference in the mean ALP concentration between groups 1 and 3 was 2.0 ±9.65 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.18).

The mean difference in the mean ALP concentration between groups 1 and 4 was -27.4 ±9.45 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups.

The mean difference in the mean ALP concentration between groups 1 and 5 was -10.8 ±17.11 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups

The mean difference in the mean ALP concentration between groups 1 and 6 was -16.6 ±3.83 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.18).

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4.14.2 COMPARING GROUP 2(CN ONLY) WITH GROUPS 3(CN+TO) AND 4(CN + CO).

The mean difference in the mean ALP concentration between groups 2 and 3 was 78.6 ±15.65 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.19).

The mean difference in the mean ALP concentration between groups 2 and 4 was 49.2± 15.85 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.19).

4.14.3 COMPARING 3(CN+ TO) WITH GROUPS 5(TO ONLY) AND 6(CO ONLY).

The mean difference in the mean ALP concentration between groups 3 and 5 was -12.8±7.46 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.20).

The mean difference in the mean ALP concentration between groups 3 and 6 was -18.6 ±5.82 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.20).

4.14.4 COMPARING GROUP 4(CN + CO) WITH GROUPS 5(TO ONLY) AND 6 (CO ONLY)

The mean difference in the mean ALP concentration between groups 4 and 5 was 16.6 ±7.66 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.21).

The mean difference in the mean ALP concentration between groups 4 and 6 was 10.8 ±5.62 at p> 0.05 indicating that there was no significant difference in the mean ALP concentration between the 2 groups (Table 4.21).

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Table 4.22. Show comparism between the mean AST for group 1 with group 2, 3, 4, 5 and 6

CONTROL Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
	2	63.2±20.25	P< 0.05
	3	27.6±24.54	P> 0.05
32.6±24.4	4	68.6±38.69	P> 0.05
	5	30.6±10.78	P> 0.05
	6	46.8±13.48	

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Table 4.23: Show comparism between the mean AST for group 2 with group 3 and 4

GROUP 2 (CN ONLY) Mean U/L.	GROUPS	TEST MEAN U/L.	p- VALUE
63.2±20.25	3	35.6±4.29	P<0.05
	4	-5.4±18.44	P< 0.05

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Table 4.24: Show comparism between the mean AST for group 3 with group 5 and 6

GROUP 3 (CN+ TO) Mean U/L.	GROUPS	TEST MEAN U/L.	P. VALUE
27.6±24.54	5	30.6± 10.78	P> 0.05
	6	46.8±13.98	P > 0.05

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Table 4.25: Show comparison between the mean AST for group 4 with group 5 and 6

GROUP 4 (CN+ CO) Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
68.6±38.69	5	30.6±10.78	P> 0.05
	6	46.8±13.48	P> 0.05

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4.15 ASPARTATE TRANSAMINASE (AST)

4.15.1 COMPARING GROUP 1 (CONTROL) WITH GROUPS 2(CN ONLY), 3(CN+TO), 4(CN + CO), 5(TO ONLY) AND 6(CO ONLY).

The mean difference in the mean AST concentration between groups 1 and 2 was -30.6±4.15 at $p<0.05$ indicating that there was a significant difference in the mean AST concentration between the 2 groups (Table 4.22).

The mean difference in the mean AST concentration between groups 1 and 3 was 5.0±6.12 at $p> 0.05$ indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.22).

The mean difference in the mean AST concentration between groups 1 and 4 was -36.0 ±15.9 at $p> 0.05$ indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.22).

The mean difference in the mean AST concentration between groups 1 and 5 was 2.0 ±15.27 at $p> 0.05$ indicating that there was no significant difference in the mean AST concentration between the 2 groups.

The mean difference in the mean AST concentration between groups 1 and 6 was -14.2 ±12.25 at $p> 0.05$ indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.22).

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4.15.2 COMPARING GROUP 2(CN ONLY) WITH GROUPS 3(CN+ TO) AND 4(CN + CO).

The mean difference in the mean AST concentration between groups 2 and 3 was 35.6 14.29 at p< 0.05 indicating that there was a significant difference in the mean AST concentration between the 2 groups (Table 4.23).

The mean difference in the mean AST concentration between groups 2 and 4 was -5.4 6.1 18.14 at p< 0.05 indicating that there was a significant difference in the mean AST concentration between the 2 groups (Table 4.23).

4.15.3 COMPARING GROUP 3(CN+ TO) WITH GROUPS 5(TO ONLY) AND 6(CO ONLY).

The mean difference in the mean AST concentration between groups 3 and 5 was -3.0 ±15.38 at p> 0.05 indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.24).

The mean difference in the mean AST concentration between groups 3 and 6 was -19.2 ±12.36 at p> 0.05 indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.24).

4.15.4 COMPARING GROUP 4(CN + CO) WITH GROUPS 5 (TO ONLY) AND 6 (CO ONLY).

The mean difference in the mean AST concentration between groups 4 and 5 was 38.0 ±31.2 at p> 0.05 indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.25).

The mean difference in the mean AST concentration between groups 4 and 6 was 21.8 ±28.18 at p> 0.05 indicating that there was no significant difference in the mean AST concentration between the 2 groups (Table 4.25).

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Table 4.26: Show comparism between the mean PCV for group 1 with group 2, 3, 4, 5 and 6

CONTROL Mean U/L	GROUPS	TEST MEAN U/L	P- VALUE
71.2±3.06	2	71.6±1.85	P> 0.05
	3	67.2±3.60	P> 0.05
	4	72.8± 2.40	P> 0.05
	5	72.6± 1.49	P> 0.05
	6	66.6± 1.85	P> 0.05

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4.16 RESULT FROM THE PACKED CELL VOLUME (PCV) ANALYSIS SHOWN PCV

4.16.1 COMPARING GROUP 1 (CONTROL) WITH GROUPS 2(CS ONLY), 3(CS + DB), 4(CS + CO), 5(CO ONLY) AND 6(CO ONLY)

The mean difference in the mean PCV concentration between groups 1 and 2 was -0.1 ±1.15 at p> 0.05 indicating that there was no significant difference in the mean PCV concentration between the 2 groups (Table 4.26)

The mean difference in the mean PCV concentration between groups 1 and 3 was 4.0 ±0.9 at p> 0.05 indicating that there was no significant difference in the mean PCV concentration between the 2 groups (Table 4.26)

The mean difference in the mean PCV concentration between groups 1 and 4 was -1.6±0.74 at p> 0.05 indicating that there was no significant difference in the mean PCV concentration between the 2 groups (Table 4.26)

The mean difference in the mean PCV concentration between groups 1 and 5 was -1.4 ±1.71 at p> 0.05 indicating that there was no significant difference in the mean PCV concentration between the 2 groups (Table 4.26)

The mean difference in the mean PCV concentration between groups 1 and 6 was 4.6 ±1.35 at p> 0.05 indicating that there was no significant difference in the mean PCV concentration between the 2 groups (Table 4.26)

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4.17 HISTOPATHOLOGICAL ANALYSIS RESULT

The rats: three in number were picked randomly for histopatological analysis. Qualitative data on histopatological analysis indicated that cyanide caused slight degeneration of the hepalocytes of the liver, necrosis of the liver and slight congestion of the kidney. These symptoms were absent with the groups treated with crude water extracts of the vegetables along with Potassium Cyanide (KCN). However there was evidence of congestion of blood vessels in both the liver and the kidney of the groups (Table 4.27). The groups treated with the vegetables alone showed little or no observable histopathology respectively.

The result from the analysis of the brain, liver and kidney are as follow:

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Table 4.27: Shows level of damage in the brain, liver and kidney in all the 6 groups

	RESULT FROM HISTOPATHOLOGY ANALYSIS			
		LIVER	KIDNEY	BRAIN
	RAT			
	B1	No visible lesion	Congestion of blood vessels	No visible lesion
GROUP 1	H1	No visible lesion	Necrosis of tubular epithelial cells	No visible lesion
	E1	No visible lesion	Mild congestion	No visible lesion
	H2	Slight degeneration of the hepatocytes of the liver	Slight congestion	No visible lesion
GROUP 2	E2	Multifocal degeneration and necrosis of the liver with loss of hepatic cords of the liver	Slight congestion	No visible lesion
	T2	No visible lesion	Mild kidney congestion	Congestion of brain
	E3	Congestion of blood vessels, mild portal lymphocytic infiltration	Congestion of blood vessels	No visible lesion
GROUP 3	B3	Focal-centriolobular hepatic necrosis	Congestion of blood vessels	Slight congestion of blood vessels
	H3	Focal-hepatic degeneration and Necrosis	Loss of tubular epithelial cells of the proximal tubules	No visible lesion
	S4	Portal-lymphocytic infiltration	Congestion of the blood	No visible lesion

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			vessels	
GROUP 4	T4	Congestion of blood vessels	No visible lesion	No visible lesion
	E4	Multifocal hepatic necrosis	Congestion of blood vessels	Blood vessels congestion
	T5	Congestion of the brain	Congestion of blood vessels	Central and portal necrosis
	B5	Mild hepatic necrosis	No visible lesion	Vacuolation and degeneration of tubular epithelial cells
GROUP 5	S5	No visible lesion	No visible lesion	No visible lesion
GROUP 6	E6	No visible lesion	Loss of tubular epithelial cells of the proximal tubules	No visible lesion
	B6	No visible lesion	Congestion of the kidney	No visible lesion
	H6	Hepatic portal necrosis and lymphatic infiltrations	No visible lesion	No visible lesion

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CHAPTER FIVE

DISCUSSION

Cyanide poisoning especially from certain diets has been implicated in outbreaks of neurological diseases. One form of cyanide -related disease is a slowly developing ataxic myeloneuropathy originally described in Nigeria (Osuntokun. 1968. 1973. 1981); the other is a sub- acute disease manifest principally by spastic paraparesis (Konzo) (Cliff et. al., 1985. Howlett et. al., 1990. Rosling. 1994; Tylleskar. al., 1992. 1993 1994). The development of these syndromes is hypothesized to depend on (a) the ammount and duration of exposure to cyanide. and (b) the ability of the body to detoxify cyanide. a function that may vary with nutritional status. Cassava – associated neurologic disease has been reported throughout southern Africa (except South Africa) and parts of central and western Africa. If cassava plant is not adequately detoxified during the processing or preparation of food. it is potentially toxic because of the release of this preformed hydrogen cyanide. The hydrogen cyanide is readily removed during processing of cassava. however. the presence of residual linamarin and its acetone cyanohydrin in cassava-based food products has the potential to cause adverse health effects in communities where cassava is a staple food. Cyanide containing plant products like cassava and sorghum form major staple food in Nigeria in particular and Africa in general. Data in this study is compatible with the findings of previous studies about the potential health hazards inherent in ingestion of cyanide or cyanide containing compounds.

For detoxification. free cyanide must be sequestered and metabolized to avoid inhibition of cytochrome c oxidase. blockage of mitochondrial electron transport and consequent energy failure. Following an acute exposure. cyanide is reportedly first trapped by methemoglobin in the form of cyanomethemoglobin (Schultz. 1984). Cyanide is converted to thiocyanate (SCN). a reaction that requires sulphane sulphur as a rate- limiting cofactor for the enzyme rhodanese (Lundquist. 1992). The concentration of sulfane sulphur is dependent on the availability of sulphur amino acids (SAA) from dietary protein (Cliff et. al., 1985). Even in protein malnutrition, available sulphur is preferentially utilized for cyanide detoxication (Sweene et. al., 1996). Cyanide also may be sequestered by albumin and metabolized to 2-aminothiazoline- 4- carboxylic acid (ATC) (Biner et. al., 1995, 1997; Lundquist et. al., 1995)

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or to cyanate (OCN) which (Swenne et al., 1996), in turn, is converted by the cysteine-containing enzyme cyanase [E.C. 3.5.5.3] ammonia and bicarbonate (Schultz, 1979). Several investigators have reported that prolonged cyanate treatment induces peripheral neuropathy in humans and rodents, and spastic paraparesis in macaques (Ohnishi et al., 1984; Shaw et al., 1974).Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the Fe3+/ Fe2+ contained in the enzyme. This causes a decrease in the utilization of oxygen in the tissues causing neurological effects such as Congestion of brain. Multi-focal degeneration and necrosis of the liver with loss of hepatic cords in the kidney as seen in group 2 and 4.

5.1 BODY WEIGHT

According to Philbrick *et al.* 1979, a reduced weight gain and decreased thyroid activity were observed in male rats given 30 mg CN kg-1 body weight day-1 as potassium cyanide in the diet for 11.5 months. This study has shown a reduction in weight gain and indeed weight loss in rats given 3mg/kg/ day as KCN by gavage for 14 days. In a study, diets deficient in methionine, vitamin B12 and iodine produced primary myelin degeneration in the spinal cord when supplemented with thiocyanate at a level of 67 mg kg-1 body weight day-1(USEPA, 1990). Adult rats in group 2 and 3 also produced slight degeneration of the hepatocytes in the liver, loss of tubular epithelial cells of the proximal tubules and congestion of the brain blood vessels. The lethal dose of HCN in mg/kg body weight are generally reported to be between 0.66mg (rabbit, i.v; USEPA, 1990) to 10-15mg for various species (rat, oral, WHO, 1965), although much larger values have been reported for mouse (oral, 598 and i.v. 184; WHO, 1965). Lethal doses of HCN in mg/kg body weight were reported for mouse, 3.7; dog, 4.0, cat, 2.0; and for cattle and sheep, 2.0 (Conn, 1979). WHO reported in 1993 that a dose of 25mg linamarin (250mg/kg body weight) fed to rats (100-120g/ body weight) caused clinical signs of toxicity including apnoea, ataxia and paresis. In the absence of methionine supplementation, 50% of these rats died within 4 hours. With adequate methionine supplementation, 10% of rats died and about 40% showed no signs of toxicity (reviewed by Oke, 1980). This study reports that rats fed with 3mg/kg cyanide by gavage daily caused clinical signs of toxicity but not

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lethal to the animals. Corchorus olitorus and Telfairia occidentalis ameliorated the effects of cyanide on body weight in group 3 196.6±34.9g and 215.7±19.2 in group 4

5.2 OCULAR AND OTHER NEUROLOGIC LESIONS

The histopathologic lesions observed in all species consisted of demyelination, especially of the optic nerve tracts and the corpus callosum. Swelling of astrocytes and myelin damage were apparent within 2days in rats injected with sodium cyanide at doses sufficient to keep the rats comatose for 225 to 260 minutes (Lessell and Kuwabara, 1974). Axonal damage, with vacuolation and loss of microtubules, also occurred. Blindness was common in cyanide treated animals and was considered to be a result of persistent anoxia in the brain. Neurologic lesions attributed to sub chronic cyanide poisoning in humans are similar to those described for experimental animals. In rats, however, the corpus callosum appears to be more sensitive than the optic nerves, whereas in humans, optic nerve damage is frequently the only central nervous system lesion (Way, 1982). Rats fed with 3mg/kg/day KCN for 14 days showed clinical signs of ocular lesion. This effect was reduced when the cyanide dosage was combined with equal amount of crude aqueous extracts of *Telfairia occidentalis* and *Corchorus olitorus*. Numerous studies have implicated cyanide as the etiologic agent in human neuropathies, including Nigerian nutritional neuropathy, tobacco amblyopia, and Leber's optical atrophy (reviewed in Towill et al., 1978). The syndrome of tropical ataxic neuropathy includes bilateral optic atrophy, nerve deafness, sensory spinal ataxia, weakness of legs, and numbness of feet (Osuntokun, 1968). This condition is believed to be due to cyanide - induced demyelination in the brain and spinal cord and is attributed primarily to consumption of the plant cassava, which contains high levels of cyanogenic glycosides (Way, 1982). Elevated plasma and urinary thiocyanate levels and demyelination of peripheral nerves, with decreased conduction velocity, were observed in patients from Nigeria with tropical ataxic neuropathy (Osuntokun, 1968; Osuntokun et al., 1970)

Cyanide poisoning from tobacco smoke has also been implicated in the occurrence of tobacco amblyopia, an optic disorder that is common in people who smoke tobacco. Tobacco smoke is known to contain cyanide, and Wilson (1965) reported that smokers have elevated levels of plasma and urinary thiocyanate. Hydroxocobalamin and cyanocobalamin, which are capable of complexing cyanide in the bloodstream, have been shown to be effective in treating tobacco amblyopia, suggesting that cyanide itself is the

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etiologic agent in this disorder (Chisholm et al. 1967). Finally, an inborn error in cyanide metabolism is thought to be the cause of Leber's hereditary optic atrophy, a condition in which bilateral vision failure occurs. Low levels of plasma thiocyanate in smokers with this condition suggest a hereditary deficiency in the ability to metabolize cyanide to thiocyanate (Wilson, 1965).

The neurologic lesions seen with all of these neuropathies are thought to be the result of cyanide -induced histotoxic anoxia (IPCS, 2000).This neurologic lesions were prominent in group CN+ *Corchorus olitorus* extract (28.6%), group CN only(67.1%) and group CN+ *Telfaria occidentalis* only (17.1%) of the total rat population Also, the acute systemic toxicity of hydrogen cyanide, sodium cyanide and potassium cyanide by instillation into the inferior conjunctival sac and oral routes have been investigated in the rabbit, the LD_{50} values in mmol/kg were 0.039 for HCN, 0.103 for sodium cyanide, and 0.121 for potassium cyanide. For all preparations, signs of toxicity appeared rapidly and death occurred within 3 to 12 min of the eye being contaminated. Cyanide concentrations in blood, serum, and various tissues were measured and the results were found to be compatible with a diagnosis of death from acute cyanide poisoning (IPCS, 2000). Thus, following their instillation into the conjunctival sac, cyanides may be absorbed across conjunctival blood vessels in amounts sufficient to produce systemic toxicity. Contamination of the eye with cyanide could be a hazardous route of exposure (Dullanty nc, 1983).

5.3 NASAL LESION

Cyanides are readily absorbed by the inhalation, oral and dermal routes of exposure. The central nervous system (CNS) is the primary target organ for cyanide toxicity. Neurotoxicity has been observed in humans and animals following ingestion and inhalation of cyanides. Cardiac and respiratory effects, possibly CNS- mediated, have also been reported. Short-term exposures to high concentrations produce almost immediate collapse, respiratory arrest and death (Hartung, 1982; USEPA, 1985). Symptoms resulting from occupational exposure to lower concentrations include pre-cordial pain and electrocardiogram (EKG) abnormalities (El Ghawabi et. al., 1975; Sandberg, 1967). Thyroid toxicity has been observed in humans and animals following oral and inhalation exposure to cyanides (Philbrick et. al., 1979, USEPA, 1984). In animal studies, cyanides have produced neuropaties, testons, fetotoxicity and teratogenic

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effects, including encephaly, encephalocele and rib abnormalities (Drakes et. al., 1986; Doherty et. al., 1982; Tewe and Maner, 1981). This study reports that rats fed with 3mg/kg/day KCN showed clinical signs of nasal lesion and nasal discharge. The symptoms were reduced when the animals were treated simultaneously with equal amount of lyophilized aqueous extracts of *Corchorus olitorus* and *Telfairia occidentalis*.

5.4 LIVER FUNCTION ENZYMES ASPARTATE TRANSAMINASE, ALANINE TRANSAMINASE AND ALKALINE PHOSPHATASE

5.4.1 ASPARTATE TRANSAMINASE

AST (glutamate oxaluacetate transaminase) is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST level to about 70± 2. In comparing the AST concentration between the control (group 1) and other groups, it was observed that there was no significant difference in the concentration between the groups.

5.4.2 ALANINE TRANSAMINASE

ALT (glutamate pyruvate transaminase GPT) is present in high concentrations in the liver and to a lesser extent in skeletal muscle, kidney and the heart. Causes of high ALT includes circulatory failure with shock, hypoxia, liver congestion secondary to congestive cardiac failure. When the mean Alanine Transaminase (mean ALT) concentration of group 1 was compared with that of group 2, it was observed that there was a significant difference $p < 0.05$ indicating that cyanide caused damage to the brain, liver and kidney of rats in this group. There was no significant difference in the mean ALT of rats in groups 3, 4, 5 and 6 when compared with the group 1(control) indicating that the effects of cyanide poisoning was ameliorated in the groups 3 and 4 treated with *Telfairia occidentalis* and *Corchorus olitorus*.

5.4.3 ALKALINE PHOSPHATASE

The ALP is a group of enzymes that hydrolyze organic phosphates at high pH. They are present in most tissues but are in particularly high concentrations in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta

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In rabbits exposed to sodium cyanide in the diet at doses of 15 mg CN /kg/day for 4 weeks or 20 mg CN /kg/day for 40 weeks, hepatic toxicity (fatty degeneration and necrosis of the liver, increased serum levels of succinate dehydrogenase, alanine aminotransferase, and alkaline phosphatase) and renal toxicity (tubular necrosis) were observed (Okolie and Iroanya, 2003; Okolie and Osagie, 1999). Neurotoxicity (myelin degeneration in the spinal cord) was observed in rats exposed at 30 mg CN /kg/day as potassium cyanide in food for 11.5 months (Philbrick et al. 1979).

In a study by Tulsawani et. al., 2005, sub-acute toxicity of potassium cyanide (KCN) in male rats following oral administration of 7.0 mg/kg (0.5 LD50) for 14 d was assessed and various hematological and biochemical indices were determined after 7 days of treatment and additional parameters like organ body weight index (OBI) and histology of brain, heart, lung, liver, kidney and spleen were performed after 14 and 21 days (recovery group) of cyanide exposure. Sub-acute exposure of KCN did not produce any significant change in body weight of the animals, OBI, hematology and the levels of blood urea, creatinine, aspartate aminotransferase, triiodothyronine (T3) and tetraiodothyronine (T4) (Tulsawani et. al., 2005). However, in KCN treated animals elevated levels of blood glucose and reduced levels of alanine aminotransferase were observed. Activities of cytochrome c oxidase in the brain and rhodanese in the liver were diminished. Reduced levels of GSH and enhanced levels of MDA in brain were observed. Increased levels of blood thiocyanate were observed in all the treatments of KCN. Additionally, KCN also produced various histological changes in the brain, heart, liver and kidney.

5.5 HISTOPATHOLOGICAL ANALYSIS

Tulsawani et. al., (2005) reported that sub-acute exposure of KCN can result in diminished activities of cytochrome c oxidase in the brain and rhodanese in the liver. Additionally, he also reported that KCN produced various histological changes in the brain, heart, liver and kidney. In group one (Control), there was no visible lesion in the liver and the brain while there was congestion of blood vessels in the kidney, necrosis of tubular epithelial cells and Mild congestion in the kidney. The rats in group two had slight degeneration of the hepatocytes of the liver, liver multi-focal degeneration and necrosis, and loss of hepatic cords in the liver with no visible lesion of the kidney in one of the rats; Slight congestion of the kidney in the three

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rats picked and no visible lesion in the brain of two rats with congestion of the brain in one of the rats.

The toxic effects of cyanide poisoning are thought to result primarily from inhibition of tissue cytochrome oxidase activity, with resulting histotoxic anoxia. In group three, congestion of blood vessels and mild portal lymphocytic infiltration, Focal centrolobular hepatic necrosis and focal hepatic degeneration were observed in the liver of the three rats selected. Congestion of blood vessels of the kidney and loss of tubular epithelial cells of the proximal tubules were observed in the kidney while there was no visible lesion in the brain. In group four, analysis indicated that there was congestion of the brain and kidney blood vessels with portal lymphocytic infiltration of the liver. (Isom and Way, 1976) found that cyanide administered with thiosulfate was lethal to mice at doses that caused no inhibition of hepatic cytochrome oxidase; however, brain cytochrome oxidase was inhibited. The brain is the organ that is most sensitive to cyanide toxicity, and death from cyanide poisoning is believed to result from central nervous system depression subsequent to inhibition of brain cytochrome oxidase activity. Although acute doses of cyanide cause cardiovascular, respiratory, and neurochemic alterations, many studies have shown that cessation of brain activity occurs prior to respiratory or cardiac arrest (Way, 1982). Pettersen and Cohen, 1985 found a similar degree of inhibition of brain cytochrome oxidase activity in C.D.1 mice administered lethal or nonlethal doses of cyanide. They also described the rapid and fairly specific changes in the central dopaminergic and γ-aminobutyric acid- ergic systems of rats and mice dosed intraperitoneally with sodium cyanide, and these changes may contribute to central nervous system depression and to the lethality of cyanide. No visible lesion was observed in the brain and kidney studied but there was a mild vacuolation and degeneration of the tubular epithelial cells of the rats selected in Group five (fed with Tellaria occidentalis only)

In group six (fed with *Corchorus olitorus* only), there was a loss of the tubular epithelial cells of the proximal tubules and hepatic portal necrosis with lymphatic infiltrations in the liver in one of the rat selected with no visible lesion in the brain, liver and kidney of the other rats. While the acute toxicity of cyanide has been thoroughly investigated for many species, relatively few experimental data exist on the effects of sub chronic and chronic cyanide exposure on animal and human model. However, the data that are available indicate that the same kinds of effects occur in humans and experimental animals. In experiments with rats (Ibrahim *et al.*, 1963; Lessell, 1971; Lessell and Kuwabara, 1974;

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Philbrick et al., 1979), cats, and monkeys (Ferraro, 1933; Hurst, 1940), selective destruction of white matter in the brain was a striking and consistent feature of poisoning from prolonged exposure to cyanide.

Neurotoxicity (myelin degeneration in the spinal cord) was observed in rats exposed at 30 mg CN kg/day as potassium cyanide in food for 11.5 months (Philbrick et al. 1979). Effects on male reproduction were severe in dogs (germ cell sloughing and degeneration, reduced spermatogenesis cycle) (Kamalu, 1993) and also observed in rats and mice in studies in which no other systemic effects were observed. Hepatic, renal, and body weight effects were reported in Wistar rats that received doses of 3.6 mg CN kg/day as potassium cyanide in drinking water for 15 days (Sousa et al. 2002a). In most of these experiments animals were injected with increasing doses of sodium or potassium cyanide for up to 132days, and the doses used were high enough to cause significant death rates from acute toxicity (Sousa et al. 2002). However, in the study by Philbrick *et al.* (1979), weanling rats exposed to low concentrations of potassium cyanide in feed had a marked decrease in weight gain as observed in group 2(188.4±15.9g), group 3(196.6±34.9g) and group 4(215.7±19.3g) but no deaths with clinical signs of toxicity. Enrly necrosis of gray and white matter was a common occurence in rats and monkeys, but repeated exposure appeared to selectively favour destruction of white matter (Philbrick et al. 1979). Administration of Potassium Cyanide at concentrations of up to 300mg/kg orally to rats and mice for 6 weeks resulted in no significant adverse effects on body weights, histopathology, or clinical pathology parameters (Okolie and Osagie, 1999). Evidence of neurologic damage was seen in the liver, brain and kidney(Okolie and Osagie, 1999; Philbrick et al. 1979). Concentrations of 100 mg/kg and greater resulted in decreased water and food consumption by rats and mice, suggesting poor palatability (Tolsawani et al., 2005). Furthermore, the epidemiologic evidence for thyrotoxic and neuro- toxic effects of cyanide after prolonged exposure in humans suggests that a difference in species sensitivity to such effects may exist between humans and rodents, and further research in this area is warranted. Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans (Oluwole et al., 2000). Data in literature indicate that long and short-term exposure to near- lethal concentrations of cyanide may produce lesions in rodents similar to those linked to chronic cyanide exposure in humans. Earlier studies have also shown higher production

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of hydrogen sulphide from cyst (e) inc than from iso-S quantities of methionine or inorganic sulphate required cyanide detoxification (Bird, 1972).

5.6 CYANIDE POISONING TREATMENT

5.6.1 DETOXIFICATION

Detoxification agents enzymatically detoxify cyanide by converting it to a relatively non-toxic product which is readily eliminated from the body. The reaction can be catalyzed by augmenting the levels of the enzyme endogenously or by supplementing the enzyme exogenously or, by providing more substrate to the enzyme, which in this case are sulfur donors (Vanlleijst et. al., 1990). The major mechanism of removing cyanide from the body is its enzymatic conversion by the mitochondrial enzyme Rhodanese (thiosulphate-cyanide sulphur transferase, (EC 2.8.1.1) to thiocyanate. Transulfuration of cyanide is also facilitated by β-mercoptopyruvate-cyanide sulphur transferase (EC 2.8.1.2) (Ballantyne, 1974). The enzymatic conversion of cyanide to thiocyanate requires a source of sulfane sulphur (divalent ionised sulphur bound to another sulphur atom) which is usually offered by thiosulfates or other biological compounds containing sulfane sulphur, like polythionates, thiosulfonates, persulfides etc. It is presumed that the sulfane sulphur binds first to the serum albumin to yield sulfane sulfur albumin complex which eventually reacts with cyanide to form thiocyanate (Westley, 1983). Exogenously administered thiosulfate usually in the form of STS (Sodium thiosulphate) would supplement this reaction rapidly. STS alone admimstered iv may be sufficient in moderate cases of cyanide poisoning while severe cases of poisoning may necessitate co-administration of other antidotes, preferably SN (Sodium nitrite) (Vanlleijst et. al., 1987). STS is contra-indicated in patients with renal insufficiency as the thiocyanate formed may cause toxicity (Vanlleijst et al., 1990). Endogenous augmentation of rhodanese has not been worked out extensively but exogenous supplementation has been reported to accelerate the transulfuration of cyanide to thiocyanate (Ehatt and Linnell, 1987). However, stability and sensitivity of the enzyme remains to be addressed. *Telfairia occidentalis* and *Corchorus olitorius* are rich sources of sulfur amino acids and both had ameliorating effect on cyanide poisoning in the groups fed with them.

5.6.2 PHYSIOLOGICAL

Oxygen appears to be a physiological antagonist against cyanide. Oxygen alone at hyperbaric pressure has slight protective effect in cyanide poisoning but it dramatically

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potentiates the protective efficacy of Sodium Nitrite (SN) and or Sodium thiosulphate (STS) (Way et. al. 1984). This protective mechanism is not yet clear because inhibition of cytochrome oxidase by cyanide does not deplete the availabilty of oxygen: only cellular utilisation of oxygen is impaired (Baskin et. al., 1992). It is presumed that intracellular oxygen tension may be high enough to cause non enzymatic oxidation of reduced cytochrome or oxygen may displace cyanide from cytochrome oxidase by mass action (Klassen, 1990). During Transulfuration there is accumulation of sulphite (SO_3^{-2}) which inhibits the progress of the reaction. It is proposed that oxygen accelerates the oxidation of sulphite; thereby enhancing cyanide detoxification (Litovitz, 1987). Transolphuration also occurs when sulphur is released from either methionine or cysteine in the presence of oxygen and there by enhancing cyanide detoxilication too.

5.6.3 BIOCHEMICAL

The compounds classified as biochemical antidotes have largely unexplained mechanism of action and are also regarded as non-specific antidotes. These compounds are usually not very effective per se but as adjuncts significantly augment the efficacy of conventional antidotes. A few chemicals belonging to this class of antidotes are:

(i) Chlorpromazine:

The potent vasodilatory action of nitrites prompted the examination of vasogenic drugs as cyanide antagonist. Chlorpromazine a neuroleptic phenothiazine was found to significantly potentiate the efficacy of SN and STS combination in cyanide toxicity (Way et. al., 1984). Its protective effect was attributed to its α- adrenergic blocking property (Kong et. al., 1983). Subsequently, the antidotal activity of chlorpromazine was related to its ability to sustain cellular calcium homeostasis and maintenance of membrane integrity by preventing peroxidation of membrane lipids (Minduh. al. et., 1988).

(ii) Other agents:

Other α -adrenergic blocking agents like phenoxybenzamine and various autonomic drugs, vasodilators such as papaverine, organic nitrates and anti-histaminic compounds have shown some antidotal efficacy in cyanide poisoning (Leung et. al., 1986). Cyanide induces respiratory cessation mediated through inhibitory action of released endorphin. Therefore, stereo-specific opiate antagonist (-) naloxone hydrochloride was found to protect against cyanide induced lethality in mice (Leung et. al., 1986). Role of neuronal

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calcium in cyanide induced neurotoxicity and beneficial effects of chlorpromazine and calcium channel blocker (diltiazem) are also well documented (Johnson et. al., 1986). The recent thrust to develop mechanistic based antidotes against cyanide poisoning has identified some new classes of lead compounds like calcium antagonists, non-hypnotic barbiturates, anticonvulsants, adrenergic blockers, antipsychotics, nitric oxide generators, other neuroprotective drugs, antioxidants, plasma expanders, glycolytic substrates, carbonyl compounds etc.

Many of these drugs have not been used clinically in humans but their results in experimental animals or in vitro are quite encouraging. Other commonly recommended antidotes are 'solution A and B' (a solution of ferrous sulfate in aqueous citric acid and aqueous sodium carbonate) and amyl nitrite. Britain's Health and Safety Executives (HSE) has recommended against the use of solutions A and B because of their limited shelf life, potential to cause iron poisoning and limited applicability (effective only in cases of cyanide ingestion, whereas the main modes of poisoning are inhalation and skin contact) (ATSDR, 2006).

5.7 GLOBAL ATTITUDE AND THE POPULAR TREATMENT

A retrospective examination of various cyanide antidotes reveals that there is no unanimity of opinion regarding the efficacy of a particular treatment regimen. This is mainly due to different experimental conditions, test protocols and species of animals employed in evaluating various antidotes. Adoption of a particular treatment in a country is dictated by various factors including the regulatory bodies and the legislations. There is no global unanimity on this issue. like Sodium Nitrite and Sodium thiosulphate combination is the drug of choice for cyanide poisoning in U.S.A. and many other countries. France and U.K. have adopted hydroxocobalamin while Germany is still continuing with 4-methylaminophenol (DMAP) and Sodium thiosulphate combination. However, SN (10 ml of 3% solution) and STS (50 ml 25% solution) combination is still the most prevalent treatment in cyanide poisoning (Vanstiliet et. al., 1987). Artificial ventilation with 100% oxygen via Ambu bag containing the contents of two ampoules of amyl nitrite (0.6 ml) is usually practiced as the first aid therapy. The use of antidote should be restricted to patients in deep coma with respiratory insufficiency. Supportive therapy of diazepam i.v.(3 x 10 mg) and 4.2% sodium bicarbonate solution to correct the convulsions and metabolic acidosis respectively have also been used in human poisoning.

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To revert excessive methaemoglobinaemia i.v. administration of 30 ml of 1% methylene blue solution is also recommended (VanHeijst et al., 1987)

5.3 ROLE OF SULPHUR-CONTAINING AMINO ACID IN CYANIDE DETOXIFICATION

Dietary cyanide exposure from cyanogenic glycosides in insufficiently processed cassava has been implicated as a contributing factor in growth retardation (Padmaja, 1996). The major defence of the human body to counter the toxic effects of cyanide is its conversion to thiosulfate mediated by the enzyme rhodanese (discovered by Lang, 1933). The enzyme contains an active disulphide group, which reacts with the thiosulphate and cyanide. The enzyme is localized in the mitochondria in different tissues and is relatively abundant, but in sites, which are not readily assessable to thiosulphate the limiting factor for the conversion of cyanide is thiosulphate. This detoxification requires sulphur donors, which are provided from sulphur-containing dietary amino acids, cysteine and methionine (Bradbury and Holloway, 1998; Rosling, 1994). In subjects who have an adequate protein component of their diet excess cysteine and methionine are not required for protein synthesis and are degraded to inorganic sulphate and excreted. Where dietary intake of protein is inadequate, the preferential use of metabolically available sulphur-containing amino acids for cyanide detoxification is also believed to hamper protein synthesis and hence contribute to growth retardation in children exposed to dietary cyanide from cassava. A deficit in height-for-age index, otherwise referred to as 'stunting' was associated with children who consumed inadequately processed cassava, however, weight-for-height and weight-for-age indices were not significantly different from children who consumed cassava which was adequately processed (Banea-Mayambu et al., 2000). This indicates that because of the preferential use of sulphur amino acids for cyanide detoxification in the human body, dietary cyanide exposure may be a factor aggravating growth retardation.

Some cassava products are eaten with soup that contains three main groups of food items. First there are various seeds and nuts that are usually ground up and used to thicken the soup, either by themselves or in a mixture of some starchy staples or okra. These are high in protein, fats and other nutrients. Second, there are leafy and fruit vegetables such as African spinach, ugwu and okra that are sources of minerals, vitamins and fiber (Okigbo, 1999). The kind of animal product used in the soup also usually depends on social status

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or income and the occasion for which the meal is prepared. Since the soup is rich in sulfur amino acids, the toxicity of any cyanide in the cassava product eaten may be minimized by the detoxifying effects of the sulfur amino acids in the animal products. Free cyanide must be sequestered and metabolized to avoid inhibition of cytochrome c oxidase, blockage of mitochondrial electron transport and consequent energy failure. Following an acute exposure, cyanide is reportedly first trapped by methemoglobin in the fonn of cyano- methemoglobin (Schultz, 1984). Cyanide is converted to thiocyanate (SCN-), a reaction that requires sulfane sulphur as a rate- limiting cofactor for the enzyme rhodanese (Lundquist, 1992). The concentration of sulfane sulphur is dependent on the availability of sulphur amino acids (SAA) from dietary protein (Cliff et. al., 1985). Even in protein malnutrition, available sulphur is preferentially utilized for cyanide detoxication (Swenne et. al., 1996). Cyanide may also be sequestered by albumin and metabolized to 2- aminothiazoline-4-carboxylic acid (AIC) (Lundquist et. al., 1995) or to cyanate (OCN-) which (Swenne et. al., 1996), in turn, is converted by the cysteine- containing enzyme cyanase (E. C. 3.5.5.3) ammonia and bicarbonate (Schultz, 1979).

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CHAPTER SIX

CONCLUSION AND RECOMMENDATION

There are diverse approaches to antagonise cyanide toxicity. However, full expression of antidotal potency of a regimen principally lies on clinical presentations and the immediate judgement.

This study has shown the potential of Corchorus olitorus and Telfairia occidentalis as safe antidotes for cyanide poisoning when administered as treatment regimen particularly when taken concomitantly with cyanide containing food item (s) like cassava in communities where this type of food is their staple. The availability of potentially safer antidotes like *Corchorus olitorus* and *Telfairia occidentalis* unveils the possibility of their value as first-line treatment, even in a complex clinical situation, where diagnosis is rapid and presumptive.

Considering the rapidity of cyanide poisoning, objective of further research should not be to replace the established antidotes completely but to augment their efficacy to a significant level or evolve new regimens with enhanced efficacy and safety which is acceptable with global consensus.

It is therefore recommended that further study be carried out to identify the actual components or molecules responsible for or associated with these potentials in each of the plant candidate. With the resurgence of interest on cyanide antidotes a more effective prophylactic or therapeutic regimen can be anticipated in near future.

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