EFFECTS OF AQUEOUS EXTRACTS OF *Vernonia amygdalina* AND *Talinum triangulare* ON INDUCED CYANIDE POISONING IN WISTAR RATS

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CERTIFICATION

This is to certify that the dissertation entitled "Effects of aqueous extracts of *Vernonia amygdalina* and *Talinum triangulare* on induced cyanide poisoning in Wistar Rats" submitted to the Department of Environmental Health Sciences, College of Medicine, University of Ibadan, for the award of the degree of Master of Public Health (MPH) in the Faculty of Public Health, is an original work carried out by AJAO, MUTIU YOMBO in the Department of Environmental Health Sciences, College of Medicine, University of Ibadan.

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DEDICATION

This research work is dedicated to Allah for the gift of life, also to the servitude of humanity based on the teachings of Prophet Muhammed (SAW) and for scholastic purposes. Also to my loving wife, Basirat for her imameasurable support and encouragement. To my precious children: Jumat, Zeenat and Yasmeen.

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ABSTRACT

There is a risk of cyanide poisoning in Nigeria through environmental exposure and consumption of improperly processed cyanide containing foods. Sulphur containing amino acids in vegetables like *Vernonia amygdalina* (bitter leaf) and *Talinum triangulare* (water leaf) have potential 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 was therefore designed to assess the effect of these two vegetables on induced cyanide poisoning in Wistar rats.

Thirty 7 weeks old Wistar rats with mean body weight of (123.97± 17.7) gm, (124.9± 16.7) gm, (141.4± 21.0) gm, (128.4± 23.6) gm, (145.0±11.1) gm, (118.5±13.1) gm and (129.1± 18.5) gm were fed on commercial rat pellets and water ad-libitum for two weeks and were randomly allocated to one control group and five treatment groups of five rats each. Lyophilized water extracts of *Vernonia amygdalina* and *Talinum triangulare* were reconstituted in water to give a concentration of 3mg/kg/day. The groups were treated with KCN and aqueous vegetable extracts both at dose of 3mg/kg/day by oral gavage as follows: Aqueous KCN (group 1); KCN and *Vernonia amygdalina* extracts (group 2); KCN and *Talinum triangulare* extracts (group 3); Vernonia *amygdalina* extracts only (group 4); *Talinum triangulare* extracts only (group 5). The control group was treated with distilled water only (group 6. Body weight, presence of ocular lesion, and nasal discharge were documented daily. Elevated blood

level of Aspartate Amino Transminase (AST) and Alanine Amino-Transminase (ALT) were used as indicators for liver damage. Histopathological changes in the brain, liver, kidney and spleen were documented. Data were analyzed using descriptive statistics, Student's t-test and ANOVA.

Slimy nasal discharge was found in 20.0% of rats in group 1 and 9.8% in group 5 only. There were no visible signs of ocular lesions in all rats. Mean values for AST were: 29.0±8.8 U/L , 33.0±5.2 U/L, 18.7±7.6 U/L, 24.7±13.3 U/L, 16.0±7.2 U/L and 20.7±6.1 U/L ($p>0.05$) for groups 1 to 6 respectively. Mean values for ALT were; 17.7±3.5 U/L, 18.0±7.0 U/L, 22.3±8.6 U/L, 22.3±5.1 U/L, 17.3±4.7 U/L and 21.3±7.1 U/L ($p>0.05$) for groups 1 to 6 respectively. Packed cell volume significantly increased in group 4 (45.2±3.3) as compared with control (37.0±4.4)($p<0.05$). Haemoglobin significantly increased in group 4 (14.0±2.3)when compared with control (12.4±0.5) ($p<0.05$). Histopathological changes observed with cyanide in group 1 was: multifocal fatty and portal lymphocytic degeneration of the liver; congestion, necrosis and glomerular cast of the kidney and splenic lymphoid depletion.

Vernonia amygdalina and *Talinum triangulare* reduced cyanide toxicity in rats implying that they have some detoxification properties. Bioassay fractionating of the vegetables is recommended to isolate and identify the molecules responsible for the activities.

Keywords: Cyanide poisoning, Wistar rats, *Vernonia amygdalina*, *Talinum triangulare*, Detoxification.

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CHAPTER ONE

INTRODUCTION

1.1 Background of study

Cyanides are produced by certain bacteria, fungi, and algae and are found in a number of foods, plants and their products. In plants, cyanides are usually bound to sugar molecules in the form of cyanogenic glycosides and defend the plant against herbivores (ATSDR, 2006). Cassava roots (*Manihot esculenta*), an important food grown in tropical countries, contain cyanogenic glycosides which are produced by over 1,000 plant species including commonly consumed vegetables (e.g. sorghum, almonds, cabbage and turnips) (Osuntokun, 1980). Cyanide is present in trace amount in tobacco as hydrogen cyanide (HCN), also in metal polishes (especially silver polish), electroplating solutions, pyrolysis of wool, metallurgical processes, industrial intermediates and the antihypertensive drug, sodium nitroprusside (Anon, 2004)

Many cyanide-containing compounds are highly toxic, but some are not. Nitriles (which do not release cyanide ions) and hexacyanoferrates (ferrocyanide and ferricyanide, where the cyanide is already tightly bound to an iron ion) have low toxicities, while most other cyanides are deadly poisonous (USEPA, 1984). Prussian blue, with an approximate formula $Fe_7(CN)_{18}$ is the blue of blue prints and is administered orally as an antidote to poisoning by thallium and radioactive caesium-137; the large ferrocyanide anion is an effective getter for heavy monovalent cations. The most dangerous cyanides are hydrogen cyanide (HCN) and salts derived from it, such as potassium cyanide (KCN) and sodium cyanide (NaCN), among others. Also some compounds readily release HCN or the cyanide ion, such as trimethylsilyl cyanide $(CH_3)_3SiCN$ upon contact with water and cyanoacrylates upon pyrolysis (Sharpe, 1976).

Many of the cyanides in soil and water come from industrial processes. The major sources of cyanides in water are discharges from some metal mining processes, organic chemical industries, iron and steel plants or manufacturers, and publicly owned

wastewater treatment facilities (USEPA, 1985). Other cyanide sources include vehicle exhaust, releases from certain chemical industries, burning of municipal waste, and use of cyanide-containing pesticides. Much smaller amounts of cyanide may enter water through storm water runoff where road salts are used that contains cyanide. Cyanide in landfills can contaminate underground water. Hydrogen cyanide, sodium cyanide, and potassium cyanide are the forms of cyanide most likely to be in the environment as a result of industrial activities (USEPA, 1985). Hydrogen cyanide is a colorless gas with a faint, bitter, almond-like odor. Sodium cyanide and potassium cyanide are both white solids with a slight, bitter, almond-like odor in damp air (USEPA, 1985). Cyanide salts and hydrogen cyanide are used in electroplating, metallurgy, organic chemicals production, photographic developing, manufacture of plastics, fumigation of ships, and some mining processes (USEPA, 1985). Hydrogen cyanide has also been used in gas-chamber executions and as a war gas. Chlorination of water contaminated with cyanide produces the compound cyanogen chloride. Four incidents of cyanide in soil resulted from disposal of cyanide-containing wastes in landfills and use of cyanide-containing road salts (USEPA, 1985). Thiocyanates are a group of compounds formed from a combination of sulfur, carbon, and nitrogen. Thiocyanates are found in various foods and plants; they are produced primarily from the reaction of free cyanide with sulfur. This reaction occurs in the environment (for example, in industrial waste).The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure (ATSDR, 2006).

Thiocyanate is a detoxification product of cyanide. Various synthetic thiocyanates have been widely used as contact insecticides since the 1930s (USEPA, 1985). Low molecular weight homologues, such as methyl, ethyl and isopropyl thiocyanates are volatile liquids sometimes employed as insecticidal fumigants. Long chain derivatives and certain esters and ethers are oily liquids marketed as dusting powders and kerosene based sprays (Barr, 1966).

Methyl isocyanate is an intermediate in the synthesis of carbamate pesticides, such as carbaryl, methomyl and temik. There is a growing market for HCN in the synthesis of methionine used in animal feed. Ammonium thiocyanate is used as a cotton defoliant and sodium thiocyanate has been applied as a weed killer (Farmer, 1977).

Determination of cyanide in the body fluids, such as saliva, urine and blood serum is insignificant due to instability of cyanide in-vitro (Barr, 1966), and very low concentrations encountered in biological materials. In this case, determinations of thiocyanate in serum and urine have been implicated. And since the concentration of thiocyanate in serum is appreciably high when compared to that of cyanide, estimation of thiocyanate in biological fluids may serve as index of toxicity (Barr, 1966).

The main route of cyanide detoxification is a pathway that utilizes thiosulphate as a substrate for the enzyme thiosulphate: cyanide sulphur transferase, better known as rhodanese (Lang, 1933). It is evident that when relatively large but not acutely toxic amounts of cyanide are administered to experimental animals, there is a corresponding increase in the concentration of thiocyanate in the tissue fluids, and in the amount excreted in the urine (Stoa, 1957). But a high instantaneous dose will rapidly lead to increase tissue levels and symptoms of intoxication, whereas, about 10mg (0.4nmol) cyanide can temporally be neutralized by a reversible reaction with the methaemoglobin fraction in the red blood cells (Lundquist *et al*., 1985). It is also reported that by a reaction with sulfan-sulfur originating from dietary sulfur amino acids, the enzyme converts the majority of a cyanide dose to the less toxic thiocyanate (SCN), which is excreted slowly in the urine (Rosling, 1988). Moreover, the conversion rate of cyanide to SCN in well nourished adults is about 50-100mg (2 – 4nmol) cyanide/24h (Schultz, 1984), and the rate-limiting step is sulfur availability and the safe dose rate of cyanide intake from dietary sources. It is probable that reduced protein intake and resulting low sulfur availability may reduce the detoxification rate. However, subjects with very low intake of protein, and hence sulfur, are able to form at least 0.5 nmol of SCN/24h (Tylleskar *et al*., 1992).

Since the major pathway for detoxification of cyanide is by its enzymic transulphuration to thiocyanate, which is excreted by the kidney. And it was reported that elimination of thiocyanate was practically wholly renal, and the elimination constants were inversely proportional to the creatinine clearances (Schultz *et al*., 1979). There are two enzyme systems responsible for the transulphuration process; thiosulphate – cyanide sulphurtransferase (rhodanese) and B – mercaptopyruvate cyanide sulphurtransferase (Sorbo, 1975). Rhodanese, found in mitochondria, and catalyses the transfer of a

sulphane sulphur atom from sulphur donors to sulphur acceptors has been implicated in the basic reaction involving detoxification of cyanide by conversion to thiocyanate (Lang, 1933). The sulphur transferases catalyze the formation; inter conversions and reactions of compounds containing sulphane sulphur atoms (Way, 1982). Overall, sulphane sulphur is derived from mercaptopyruvate sulphur transferase, and the various form of sulphane sulphur is interconverted by rhodanese. The sulphane carrier that transports the sulphur formed is plasma albumin; the sulphane sulphur – albumin complex then reacts with cyanide. Binding is sufficiently firm and pharmacokinetic studies indicate that the conversion of cyanide to thiocyanate is predominantly in the central compartment, with a volume of distribution approximating to that of blood volume (Way, 1982). Thus, it is possible that the plasma albumin sulphane complex is a primary cyanide detoxification buffer in normal metabolism (Way, 1982). Other minor detoxification pathways for cyanide include: exhalation in breath as HCN and carbon dioxide from oxidative metabolism and erythrocytes have been reported to have high affinity for cyanide, and there is a rapid uptake of plasma cyanide by erythrocytes (Schultz, 1984). These efficient detoxification processes prevents long-term bioaccumulation of cyanide. Thus, if acute exposure is to a sub lethal dose of cyanide, this may lead to the development of signs of toxicity, but as detoxification proceeds, these signs will ameliorate and disappear, and cyanide will be excreted as thiocyanate without bioaccumulation.

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 of body 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).

1.2 Rationale for the study

Plants containing cyanoglycosides are the main source of cyanide exposure for individuals who are not occupationally exposed to the chemical. Foods containing cyanogenic glycosides form the staple food in several communities in Nigeria, thereby; general population is exposed to cyanides primarily by ingestion of the staple food (Osuntokun, 1981). Also to a lesser degree, by inhalation from combustion or pyrolysis of certain materials under oxygen-deficient conditions such plastics, exhaust of internal combustion engines and tobacco smoke (Anon, 2004). Cyanide is extremely toxic and fast acting poison; however, it can be detoxified to a certain extent in human body (Clark *et al*., 1991). Since cassava can withstand drought, it is sometimes 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 is important. Vegetable species stems are noted for 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).

1.3 Objective of the study

The objective of this study is to determine the effectiveness of *Vernonia amygdalina* (Bitter leaf) and *Talinum triangulare* (Water leaf) in the detoxification of cyanide using rats as a model.

1.4 Specific objectives

The specific objectives were to;

i. Reproduce the toxic effect(s) of cyanide in rats

ii. Investigate the efficacy of *Vernonia amygdalina* (Ewuro) and *Talinum triangulare* (Gbure) in counteracting the toxic effect of cyanide intoxication in the animal models.

1.5 Limitation of the study

i. The inability of obtaining the sulphur containing amino acids (methionine and cystein) as standard references.

ii. The inability to monitor plasma levels of cyanide in experimental animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of cyanide

Cyanide is any chemical compound that contains the cyano group (C≡N), which consists of a carbon atom triple-bonded to a nitrogen atom. Inorganic cyanides are generally salts of the anion CN^-. Of the many kinds of cyanide compounds, some are gases; others are solids or liquids. Those that can release the cyanide ion CN^- are highly toxic. 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 produced by the combustion or pyrolysis of certain materials under oxygen-deficient conditions. For example it can be detected in the exhaust of internal combustion engines and tobacco smoke (Baud *et al*. 1991). Certain plastics, especially those derived from acrylonitre, release hydrogen cyanide when heated or burnt (Baud *et al*. 1991). 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 peas 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.

2.2 Sources and uses of cyanide

2.2.1 Sources and potential exposure

Cyanide enters air, water, and soil from both natural processes and industrial activities. Airborne cyanide is generally far below levels that would cause concern.

Cyanide in air

In air, cyanide is present mainly as gaseous hydrogen cyanide. A small amount of cyanide in air is present as fine dust particles (Dinca *et al*., 1972). This dust eventually settles over land and water.Cyanide is used in a number of industries and is found at low levels in air from car exhaust (Dinca *et al*., 1972).

Rain and snow help remove cyanide particles from air. The half-life (the time needed for half of the material to be removed) of hydrogen cyanide in the atmosphere is about 1–3 years (Dinca *et al*., 1972). Most cyanide in surface water will form hydrogen cyanide and evaporate. However, the amount of hydrogen cyanide formed is generally not enough to be harmful to humans. 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 µ g/cigarette for mainstream (inhaled) smoke, and from 0.006 to 0.27 µ g/cigarette for side stream smoke. 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 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 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 patina 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).

Cyanide in water

Some cyanide in water will be transformed into less harmful chemicals by microorganisms (plants and animals of very small size), or will form a complex with metals, such as iron (ATSDR, 2006). . Cyanide is able to passes through soil into underground water. Less is known about what happens to thiocyanate when it enters the environment. 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 (ATSDR, 2006).

Cyanide in soil

In soil and water, thiocyanate is changed into other chemical forms by microorganisms (ATSDR, 2006). Cyanides are fairly mobile in soil. Once in soils, cyanide can be removed through several processes. Some cyanide compounds in soil can form hydrogen cyanide and evaporate, whereas some cyanide compounds will be transformed into other chemical forms by microorganisms in soil. Consequently, cyanides usually do not seep into underground water. However, cyanide has been detected in underground waters of a few landfills and industrial waste disposal sites. At the high concentrations found in some landfill leachates (water that seeps through

landfill soil) and in the wastes stored in some disposal sites, cyanide becomes toxic to soil microorganisms (WHO, 2004).

Cyanide in plants

Cassava accounts for 41.5 per cent of the food consumed in Western States of Nigeria, 53 per cent in Midwestern and 45 per cent in East Central. 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 (Osuntokun, 1980). Cassava seems initially to have been adopted with caution because of its toxicity but the various advantages that the cassava crop has over the indigenous yams and other staples enhanced its rapid adoption. Cassava plant including the storage root contains linamarine and taxiphillin respectively, which break down upon disruption of the plant cells to form hydrogen cyanide. Since it can withstand drought, it is sometimes a nutritionally strategic famine reserve crop in areas of unreliable rainfall (Osuntokun, 1980). However in very small amounts, cyanide is a necessary requirement in the human diet as prosthetic group of cyanocobalamine (Vitamin B12) (Osuntokun, 1980). 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 (1,900–4,600 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 (ATSDR, 2006).

Cyanide is extremely toxic to humans. Chronic (long-term) inhalation exposure of humans to cyanide results primarily in effects on the central nervous system (CNS) (ATSDR, 2006). Other effects in humans include cardiovascular and respiratory effects, an enlarged thyroid gland, and irritation to the eyes and skin (ATSDR, 2006). No data are available on the carcinogenic effects of cyanide in humans via inhalation (ATSDR, 2006). Animal studies have suggested that oral exposure to cassava (a cyanide-containing vegetable) may be associated with malformations in the fetus and low foetal body weights (ATSDR, 2006). 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.. 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).

2.2.2 Uses of cyanide

Medical uses

The cyanide compound sodium nitroprusside is occasionally used in emergency medical situations to produce a rapid decrease in blood pressure in humans; it is also used as a vasodilator in vascular research. During World War I, a copper cyanide compound was briefly used by Japanese physicians for the treatment of tuberculosis and leprosy (Van Heijst *et al*., 1987)

Fishing

Cyanides are illegally used to capture live fish near coral reefs for the aquarium and seafood markets. 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, needle less 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 (Bedding *et al*., 1982). 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 (Bedding *et al*., 1982).

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).

Execution and Notable Cyanide Deaths

Cyanides have been used as poison many times throughout history. The most infamous application was the use of hydrogen cyanide pellets by the Nazi regime in Germany for mass murder in some gas chambers during the Holocaust (Van Heijst *et al*., 1987).

Cyanides have been used (as in the case of Grigori Rasputin) for attempted murder, and for judicial execution in some parts of the United States (Van Heijst *et al*., 1987)

Some notable persons who committed suicide by cyanides (either cyanide salt or hydrogen cyanide) are Eva Braun, Wallace Carothers, Odilo Globocnik, Joseph Goebbels, Hermann Göring, Heinrich Himmler, Adolf Hitler (in combination with a gunshot), Günther von Kluge, Erwin Rommel, Alan Turing and the Liberation Tigers of Tamil Eelam. The mass suicide/murder of The People's Temple in Jonestown was done with cyanide poisoning (Van Heijst *et al*., 1987).

Other uses

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 patina 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 (Baud *et al*. 1991).Cyanide is also used in jewellery-making and certain kinds of photography (ATSDR, 1993). Although generally thought to be toxic, cyanide and cyanohydrins have been demonstrated to increase germination in various plant species (ATSDR, 1993).

2.3 Mechanism of cyanide toxicity

The cyanide anion is an inhibitor of the enzyme cytochrome c oxidase in the fourth complex of the electron transport chain (found in the membrane of the mitochondria of eukaryotic cells). It attaches to the iron within this protein. The binding of cyanide to this cytochrome prevents transport of electrons from cytochrome c oxidase to oxygen.

As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy. Tissues that mainly depend on aerobic respiration, such as the central nervous system and the heart, are particularly affected (Isom and Way, 1976).

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 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 (Isom and Way, 1976). 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 (Rosling, 1994). If the HCN exceeds the limit an individual is able to detoxify or tolerate, death may occur while smaller sub- lethal 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 (Rosling, 1994).

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 (Rosling, 1994). 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 (Tylleskar *et. al*., 1993). 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 (Tylleskar *et. al*., 1993). Human cassava eating population showed opthalmological and neurological symptoms, which are associated with exposure to HCN (Tylleskar *et. al*., 1993).
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. 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 (Oke, 1980). 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 (Oke, 1980).

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) (Farmer, 1977). Therefore, some of these vegetables are the cheapest and most readily available sources of important proteins, vitamins and essential amino acids.

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) (JECFA, 1993). 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 body 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- fatal amount of cyanide cause acute intoxication (US EPA, 1984).

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, treatment provided before sampling (if any), and sample handling between collection and analysis (Yeaoh and Braitberg, 2004).. In adults, the blood cyanide level that is regarded as "toxic" is generally considered to be $\geq$ 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 and Braitberg, 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 younger than 15 years (American Academy of Paediatrics, 2000). In children, as in adults, the majority of fire-related deaths are attributed to smoke inhalation rather than burns (American Academy of Paediatrics, 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 (Barillo and Goode, 1994). 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-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 and Goode, 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 109 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 µmol/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. Cyanide is used in a number of industries and is found at low levels in air from car exhaust. Cyanide is extremely toxic to humans. Chronic (long-term) inhalation exposure of humans to cyanide results primarily in effects on the central nervous system (CNS). Other effects in humans include cardiovascular and respiratory effects, an enlarged thyroid gland, and irritation to the eyes and skin. No data are available on the carcinogenic effects of cyanide in humans via inhalation. Animal studies have suggested that oral exposure to cassava (a cyanide-containing vegetable) may be associated with malformations in the fetus and low fetal body weights.

2.4 Properties of cyanide

2.4.1 Physical properties

Cyanide is present in a number of compounds such as hydrogen cyanide, sodium cyanide, and potassium cyanide. Hydrogen cyanide is a colourless gas or liquid with a faint, bitter almond odour. The odor threshold for hydrogen cyanide is 0.58 parts per million (ppm). The chemical formula for hydrogen cyanide is HCN, and the molecular weight is 27.03 g/mol. The vapor pressure for hydrogen cyanide is 264.3 mm Hg at 0 °C, and its log octanol/water partition coefficient (log K_{ow}) is 0.66. Sodium cyanide and potassium cyanide are both colourless solids that possess the slight odour of bitter almonds (USEPA, 1997; ATSDR, 2006).

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 25.6^0C (Verschueren, 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.*, 1994).

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:

$NaCN + H_2O \rightarrow HCN + NaOH$

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:

- 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 cyanides 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:

$RCH{=}CH_2 + HCN \rightarrow RCH\ (CN)\ CH3$

Metal catalysts are required for such reactions.

2.4.2 Chemical properties

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

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 complexes form insoluble precipitates with iron, copper, nickel, manganese, lead, zinc, cadmium, tin and silver. 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 aluminum, 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) Sulphuration

Cyanide reacts with some sulfur species to form less toxic thiocyanate. Potential sulfur sources include free sulfur and sulfide minerals such as chalcopyrite (CuFeS2), chalcocite (Cu2S) and pyrhotite (FeS), as well as their oxidation products, such as polysulfides and thiosulfate (ICMI, 2006).

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).

2.5 Effects of cyanide exposure on the environment and human

2.5.1 Effects on the environment

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).

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 and 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 and Maduagwu, 2000).

Water

Cyanides, in form of, 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 (US EPA, 1993). 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, 1993). 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 (Bedding *et al*., 1982). In a survey in 1987 of over 35 drinking-water supplies, the quarterly median cyanogen chloride concentrations in drinking water ranged from 0.45 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 unweathered 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 15–400 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 gari 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).

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 mortality, 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 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.

Birds

Reported oral Lethal Dose 50units 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, laboured 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).

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 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).

2.5.2 Effects on human

Natural occurrence

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 (Ermans *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*., 1986).

Hydrogen cyanide is ubiquitous in nature. It is found in the stratosphere and non-urban troposphere (US EPA, 1993). It is released into the atmosphere from biomass burning, volcanoes, and natural biogenic processes from higher plants, bacteria, algae, and fungi (ATSDR, 1997; Fiksel *et al*., 1981; Way, 1982; Cicerone & Zellner, 1983; Li *et al*., 2000;). An estimate of the amount of cyanide released to the environment from natural biogenic processes is not available (ATSDR, 1997). In air, cyanide is present as gaseous hydrogen cyanide, with a small amount present in fine dust particles (WHO, 2004).

Anthropogenic Sources

Hydrogen cyanide is produced by the combustion or pyrolysis of certain materials under oxygen-deficient conditions. For example it can be detected in the exhaust of internal combustion engines and tobacco smoke. Certain plastics, especially those derived from acrylonitrile, release hydrogen cyanide when heated or burnt (ATSDR, 1997).

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, 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; sidestream: mainstream ratios of hydrogen cyanide concentrations ranged from 0.06 to 0.50 (ATSDR, 1997).

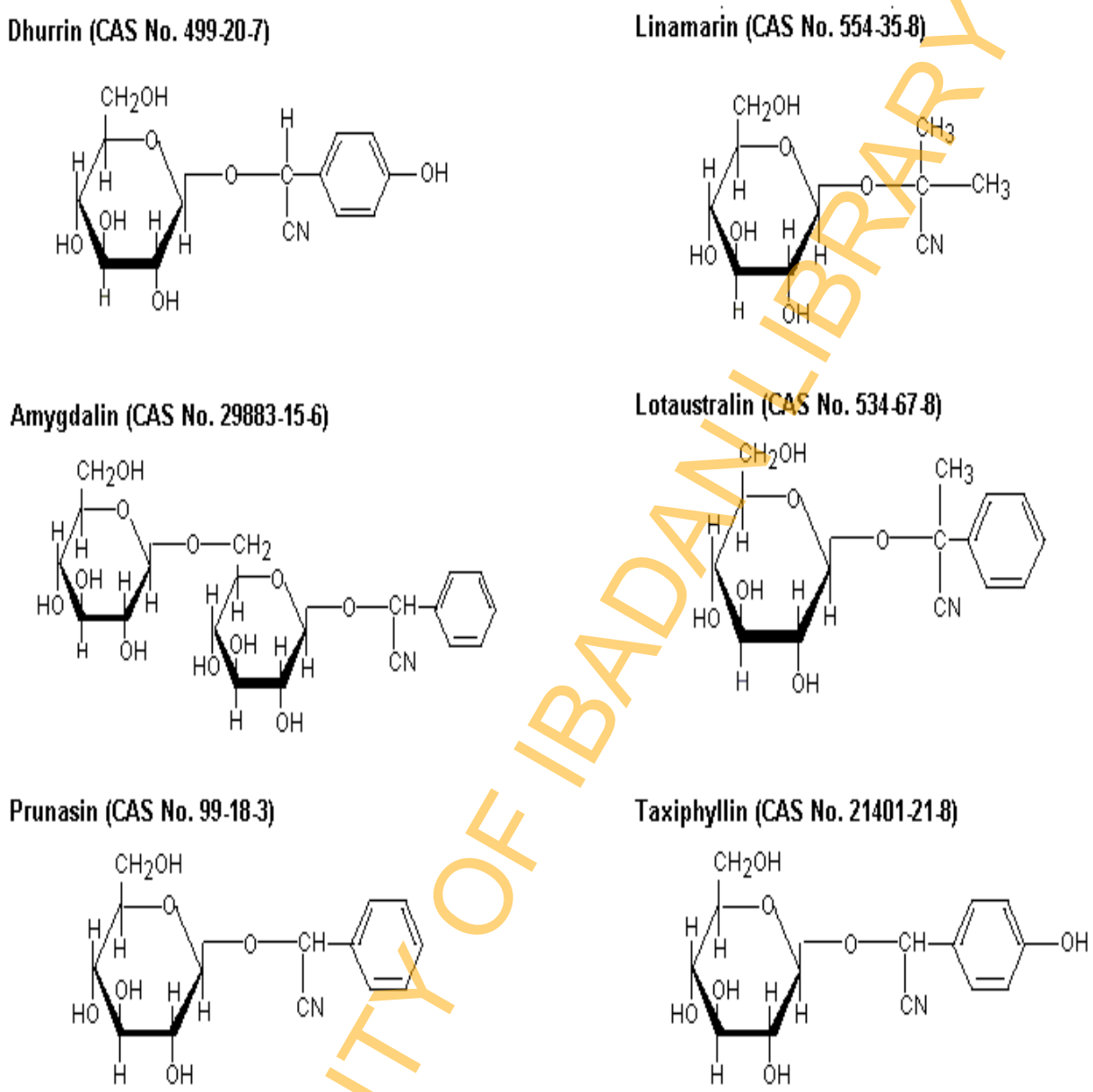

Figure 2.1: Cyanogenic glycosides in edible plants (JECFA, 1993)

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 sidestream 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 and Kanno 1987; IPCS, 2000). In 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, 1985).

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
Home-made 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
Lima beans from Puerto Rico (black)	2900
Lima beans from Burma (white)	2000

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

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 "gari," 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 and 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) (IPCS, 1992).

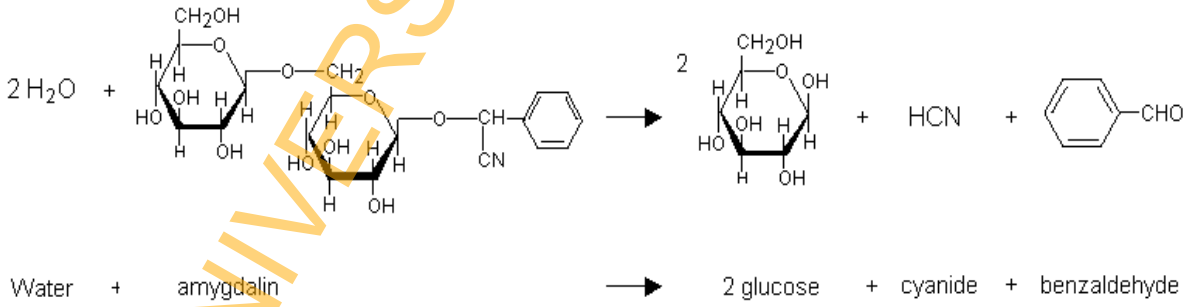

Figure 2.2: Hydrolysis of amygdalin

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, 1995). 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 and 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 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. 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 0.54-mg/kg-body weight, and the average absorbed dose at the time of death has been estimated at 1.4-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 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 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 µg/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, 2004).

2.6 Metabolism and kinetics of cyanide in humans and experimental models

2.6.1 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 (ATSDR, 1997; Landahl and Herrmann, 1950). 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 (ECETOC, 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.

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^{-4} cm/h (Dugard, 1987).

2.6.2 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; ATSDR, 1997; Ballantyne, 1983; 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 (<140 µg/litre) and other tissues (<0.5 mg cyanide/kg) of humans without known occupational cyanide exposure (Feldstein and 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 (Feldstein and Klendshoj, 1954). After cessation of exposure, plasma cyanide levels tend to return to normal within 4–8 h (Feldstein and Klendshoj, 1954; Ansell and 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, 1983) have been reported. For a given exposure route, whole blood and serum cyanide levels are quite similar for different species (Ballantyne, 1983).

2.6.3 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 (ATSDR, 1997). Dogs have a lower overall activity of rhodanese than monkeys, rats, and rabbits (ATSDR, 1997).

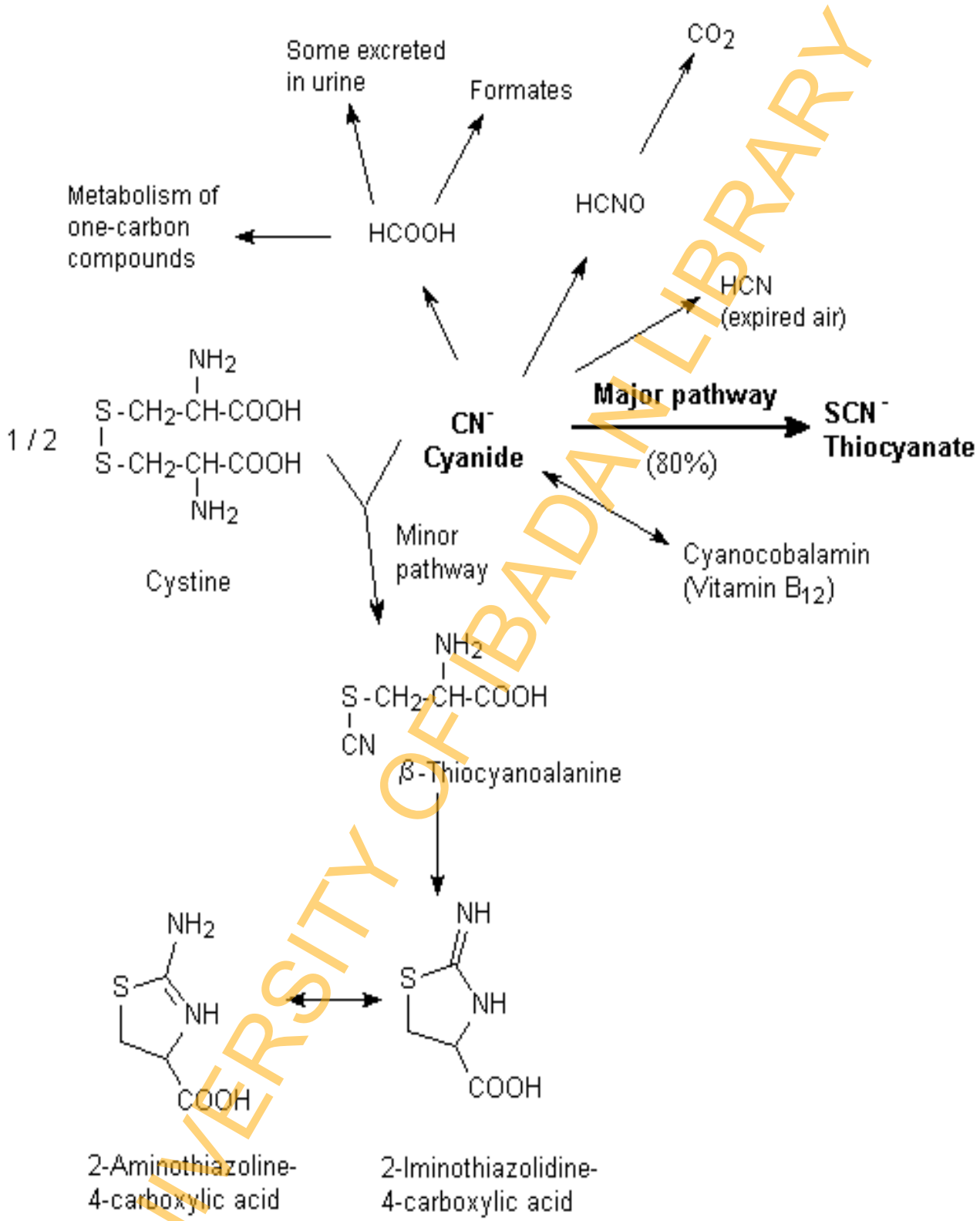

Figure 2.3: Basic processes involved in the metabolism of cyanide (ATSDR, 1997)

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_{12}a) to yield cyanocobalamin (vitamin B_{12}) (Boxer and Rickards, 1952) and the non-enzymatic combination of cyanide with cystine, forming 2-iminothiazoline-4-carboxylic acid, which appears to be excreted without further change.

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; JECFA, 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, 1982), which is considerably slower than in small rodents (Schubert and Brill, 1968) or dogs (Lawrence, 1947).

2.7 Potential health effects in humans

2.7.1 Effects of short-term (Acute) exposure

Inhalation:

Potassium cyanide is a solid, which does not form a vapour at room temperature. However, inhalation to 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 (0.6-4.5 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, 1974).

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 and Rumack, 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 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 (Basu, 1983). 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) (Gosselin *et. al.*, 1984). 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, 2004).

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).

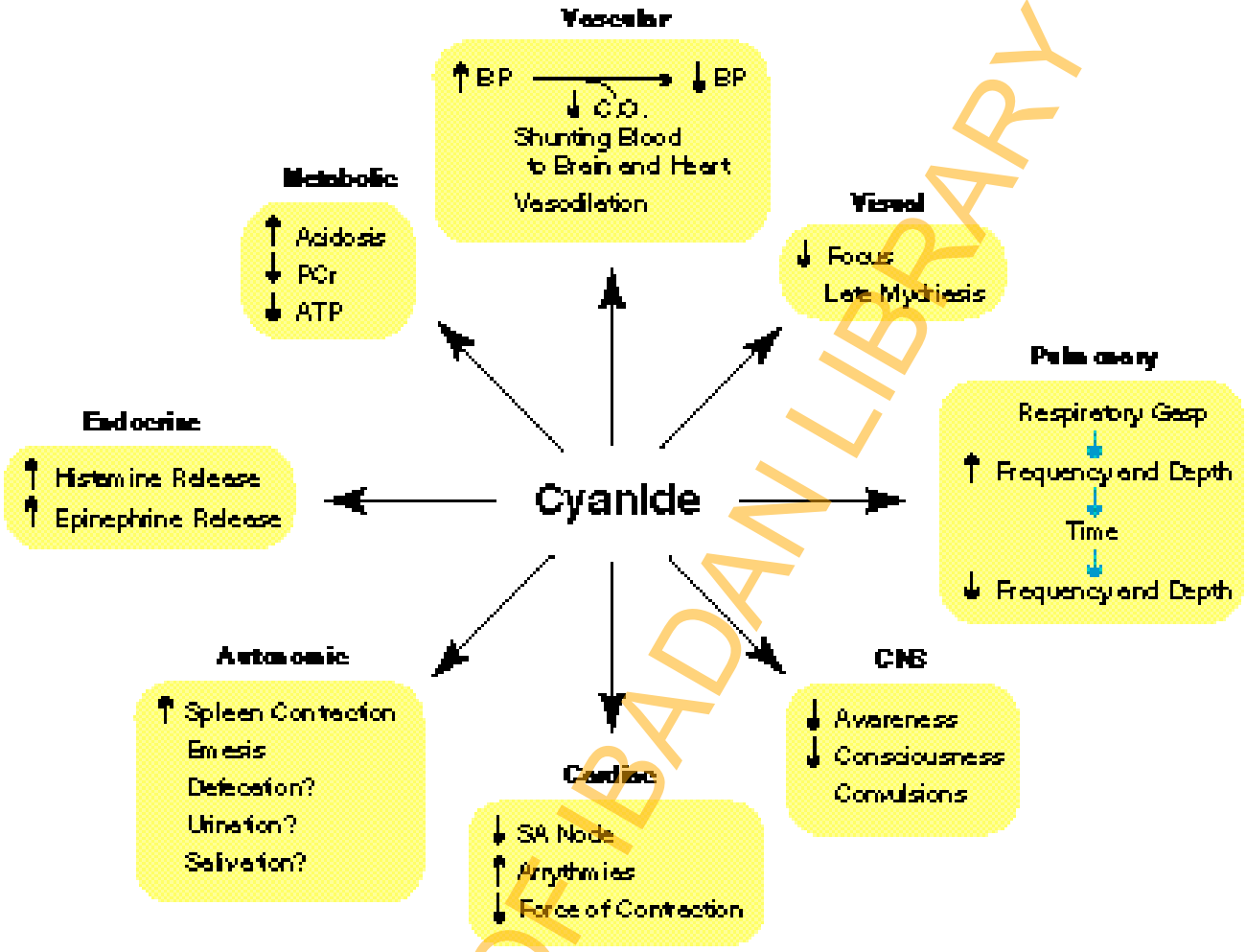

Figure 2.4: Cyanide toxicity pathways

Baskin *et. al.* 1998

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

2.7.2 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 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 34 matched controls. Headaches were more frequently reported in exposed workers (Kumar et. al., 1991).

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 and Obasi, 1991).

Lungs/Respiratory System:

Two limited studies suggest that long-term cyanide exposure may be associated with labored 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*., 1991). 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 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 and Obasi, 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 34 employees exposed to unspecified concentrations of hydrogen cyanide, while engaged in case hardening and electroplating for 2-20 years (Kumar *et al.*, 1991) 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 areas. In tests done 7-30 months after the last exposure, the thyroid-stimulating hormone was 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, 1983).This enzyme is 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 (Gosselin *et al*., 1984).

2.7.3 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), but has also been observed in Mozambique,

Tanzania, Central African Republic and Cameroon (Ministry of Health, Mozambique, 1984; Lantrum *et al*, 1988; Howlett *et al*., 1990; Tylleskar *et al*., 1992). 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 *et al.*, 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.

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 (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 40 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 (Rosling, 1988).

2.8 Kinetics of cyanide, health effects, treatment of poisoning and antidotes in human

2.8.1 Kinetics of cyanide and health effects

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

The effects of acute cyanide exposure are dominated by central nervous system and cardiovascular disturbances (ATSDR, 1993). Typical signs of acute cyanide poisoning include tachypnoea, headache, and vertigo, lack of motor coordination, weak pulse, cardiac arrhythmias, vomiting, stupor, convulsions, and coma (Way, 1982; Ballantyne, 1983). Pathological findings may include tracheal congestion with haemorrhage, cerebral and pulmonary oedema, gastric erosions, and petechiae of the brain meninges and pericardium (Way, 1982). 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, 1993). 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 (LC50 or LD50). The LC50 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 LD50 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 LD50 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 (ATSDR, 1993). 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-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 (Hartung, 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 amino thiazoline 4 carboxilic acid with other minor metabolites (ATSDR, 1989).

2.8.2 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, 1982; Isom and Borowitz, 1995). 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 and Borowitz, 1995).

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 and Bodansky 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 (Van Heijst *et al*., 1987). However, the efficacy of amyl nitrite as methemoglobin inducer remained disputed on account of its inability to generate methemoglobin greater than 6%, while about 15% is required to challenge one LD50 dose of cyanide (Van Heijst *et al.*, 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 and Froelich. 1985).

(ii) Sodium nitrite:

Sodium nitrite (SN) is the most prevalent drug of choice for cyanide poisoning (Chen and Rose, 1952). When given intravenously (i.v.) it takes about 12 min to generate approximately 40% of methemoglobin (Van Heijst *et al*., 1987). Inspite of this delay in inducing a significant level of methemoglobinemia, a reasonable protection offered by SN can be ascribed to its vasodilatory property (Van Heijst et al., 1987). A serious drawback with SN is that (intra venous) i.v 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 haemolytic reactions (Van Heijst *et al*., 1987).

(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 (Kiese and Munch, 1950). However, there are differences in individual susceptibility to DMAP, which may result in an undesirable level of methemoglobinemia even after normal therapeutic doses (Van Heijst *et al.*, 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 that was endowed with an anticonvulsive property (Wood and Peesker, 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.*, 1991). Although, this regimen minimised 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 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 ρ-aminopropiophenone (PAPP), ρ -aminooctanoylphenone (PAOP), ρ -nitrosopropiophenone (PNPP) and ρ -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.*, (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 LD90 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 (Van Heijst *et al.*, 1987).

(ii) Hydroxocobalamin (Vitamin B 12a):

This agent is perhaps the most promising cyanide antidote used in human toxicology (Van Heijst *et al.*, 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. 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. A recorded side effect of hydroxocobalamin includes anaphylactoid reactions and acne.

(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, 1982). Sodium pyruvate was reported to effectively challenge acute cyanide poisoning in mice (Schulz, 1984). Another ketocarboxylic acid like ketoglutaric acid (KG) is currently being pursued widely as a cyanide antidote (Dulaney *et al*., 1991). Protective effect of KG was also observed against cyanide induced convulsions in mice (Yamamoto, 1990). KG either alone or in combination with SN and/or STS attenuated toxicity in mice exposed to cyanide through different routes (Bhattacharya *et. al*., 1991) Prophylactic or therapeutic ability of KG was also shown to be augmented by oxygen (Delhumeau *et al*., 1994). Cyanide induced histotoxic hypoxia was reversed by KG which was found to be more effective than cobalt edetate and sodium pyruvate (Delhumeau *et al*., 1994). 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 KG is sold as an over-the counter nutritional supplement (Klaire Laboratories, San Marcos, CA) (Dulaney *et al*., 1991).

Detoxification

Under this group those agents are listed which 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. 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-mercaptopyruvate-cyanide sulphur transferase (EC 2.8.1.2) (Ballantyne, 1974). The enzymatic conversion of cyanide to thiocyanate requires a source of sulfane sulphur (a 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 *et al.*, 1983). Exogenously administered thiosulfate usually in the form of STS would supplement this reaction rapidly. STS alone administered i.v. may be sufficient in moderate cases of cyanide poisoning while severe cases of poisoning may necessitate co-administration of other antidotes, preferably SN (Van Heijst *et al*., 1987). STS is contra-indicated in patients with renal insufficiency as the thiocyanate formed may cause toxicity (Van Heijst *et al*., 1987). Endogenous augmentation of Rhodanese has not been worked out extensively but exogenous supplementation has been reported to accelerate the transulfuration of cyanide to thiocyanate (Bhatt and Linnell, 1987). However, stability and sensitivity of the enzyme remains to be addressed.

Physiological

Oxygen appears to be a physiological antagonist. Oxygen alone at hyperbaric pressure has slight protective effect in cyanide poisoning but it dramatically potentiates the protective efficacy of SN and/ or STS (Way *et al.*, 1984). This protective mechanism is not yet clear because inhibition of cytochrome oxidase by cyanide does not deplete the availability 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).

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). 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 (Maduh *et. al*., 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. 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 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 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).

2.8.3 Global attitude and the popular treatments

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 SN and STS combination is the drug of choice for cyanide poisoning in U.S.A. and many other countries, France and U.K. have adopted kelocyanor while Germany is still continuing with DMAP and STS combination. However, SN (10 ml of 3% solution) and STS (50 ml 25% solution) combination is still the most prevalent treatment in cyanide poisoning (Van Heijst *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. To revert excessive methaemoglobinaemia i.v. administration of 30 ml of 1% methylene blue solution is also recommended (Van Heijst *et al*., 1987)

2.8.4 Role of sulphur-containing amino acids in cyanide detoxification

Dietary cyanide exposure from cyanogenic glycosides in insufficiently processed cassava has been implicated as a contributing factor in growth retardation. 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 accessible 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, 1988; 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 them 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. The kind of animal product used in the soup also usually depends on social status 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 form 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 prefentially utilized for cyanide

detoxication (Swenne *et. al*., 1996).Cyanide may also be sequestered by albumin and metabolized to 2- aminothiazoline-4-carboxylic acid (ATC) 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.

2.8.5 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).

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.

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 (Fasuyi and Aletor, 2005). They add flavor, 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 *Vernonia amygdalina*

Botanic description

Vernonia amygdalina is a bushy shrub or well-formed tree up to 7 m in height. Bark light grey or brown, rather rough and longitudinally flaking; branches brittle. Leaves lanceolate to oblong; up to 28 x 10 cm, but usually about 10-15 x 4-5 cm. Leathery, medium to dark green, with or without sparse hairs above, with fine, soft, pale hairs below and conspicuous net-veining; apex and base tapering, base always almost symmetric, margin entire or very finely toothed; petiole usually very short but may be 1-2 cm long. Flower heads thistle-like, small, creamy-white, sometimes slightly touched with mauve, about 10 mm long, grouped in dense heads, axillary and terminal, forming large flat clusters about 15 cm in diameter but not conspicuous; sweetly scented, especially in the evening. Fruit a small nutlet, with minute glands and bristly hairs on the body and a long tuft of bristly hairs at the top. The genus was named in honour of an English botanist, William Vernon, traveller and plant collector in North America in the 17th century (USDA, 2010)

Vernonia amygdalina (bitter leaf) is a thick shrub often found in savanna and forest margins, widely distributed throughout the tropical Africa. It is often planted and is frequently items of market-merchandize, for its many uses such as quinine substitutes for fever, laxatives, expectorant, stomachic and gastrointestinal troubles.

The leaves are bitter. Bitterness can be abated by boiling or in the young leaves by soaking in several changes of water. They are held to be anti-scorbutic and are added to soups or eaten as spinach (Singha, 1965). The plant is cultivated for its leaves which can be made into sauce and eaten with cooked tapioca (fufu) (Deighton, 1957). The leaves are taken in Nigeria as an appetizer, digestive tonic and widely used for fevers and are known as quinine substitute (Deighton, 1957).

The leaves, although rather bitter to taste, are eaten as raw vegetables. ‘Chewsticks’ from the roots and twigs are regarded as an appetizer (Katende *et al*,1995). An infusion from the roots is given to children suffering from infection by a trematode (Enterobius vermicularis). A cold infusion of the root bark, together with other plants, is given in daily doses to treat bilharzia. The bark and root are taken as a tonic by people suffering from fevers; leaves are also pounded, the juice extracted and drunk for fever. The leaves

are pounded and mixed with warm water for bathing to treat spots on the skin and nausea (Kokwaro, 1976)

Ecology and geographic distribution

Vernonia amygdalina is found in Afro-montane rainforest, undifferentiated afro-montane forest (broadleaved forest, mixed Podocarpus forest) and dry single-dominant afro-montane forest (Juniperus and Juniperus-Olea); also in secondary montane evergreen bushland and sometimes forming clumps in upland wooded grassland. Elsewhere also in lowland humid rangeland, savannah and riverine fringes, often associated with termite mounds (USDA.gov, 2010).

Native : Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Liberia, Malawi, Mali, Mauritania, Niger, Nigeria, Rwanda, Sao Tome et Principe, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Yemen, Republic of, Zambia, Zimbabwe (USDA.gov, 2010).

Scientific Classification of *Vernonia amygdalina*

Kingdom: Plantae

Phylum: Spermatophyta

Class: Dicotyledones Genus: Asteraceae

Order: Asterales Genus: *Vernonia amygdalina*

Source: (USDA.gov) (Plant identifier, 2010)

Figure 2.5: *V. amygdalina* in its natural habitat: (Source: United States Department of Agriculture; USDA.gov – Plant identifier, 2010)

Table 2.2: Proximate, phytochemical and mineral compositions of aqueous extracts of *V. amygdalina* leaves

Moisture Content (%)	Dry Matter (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate (%)
8.82	91.18	21.70	2.74	10.96	9.88	54.72

Alkaloids (%)	Saponin (%)	Flavonoids (%)	Anthocyanic (%)	HCN (mg/kg)	Sterols (%)	Tannin (%)
1.52	0.20	0.04	0.02	6.22	0.04	0.28

Ca mg/100g	Mg mg/100g	K mg/100g	Na mg/100g	P mg/100g	Fe mg/kg	Zn mg/kg	Pb mg/kg	Cu mg/kg	Cd mg/kg	Cr mg/kg)
10.80	40.50	375	30	410	34	5.20	0.82	12.46	0.05	0.01

Source: Dike (2010)

Amino Acid Profile of Vernonia amygdalina.

Analysis of the amino Acid contents of *Vernonia amygdalina*

Types of amino acids analysis (mg/100 g)

Ihiamine	170.00
Pyrdoxine	2.06
Ascorbic acid	20.49
Glycine	4.63
Cysteine	1.84
Hydrolysate Casein	96.99
Nicotinamide	1.65

Source: (Alabi *et al*, 2005)

2.9.2 *Talinum triangulare*

Botanic description

Talinum triangulare (water leaf) is an erect fleshy-leafed herb of open ground and in forest clearings. It is very easily propagated by cuttings and by seed. The plant is purple-flowered, a white flowered mutant gives pink-flowered f1 offspring which on selfing show a simple 1-2-1 Mendelian ratio in the f2 generation, suitable, because of the quick growing short life-cycle, for demonstrating simple genetics (Okigbo, 1967). It is used to soothe inflammations, as a diuretic anti-emetic application and remedy for stomach troubles (Bouquet, 1969).The plant is sold in markets and eaten cooked as a potherb and in soups or raw in salad, as condiments in sauces (Ainsle, 1937). It is rich in mineral salts and amino acids and has anti-scorbutic properties (Busson, 1965). A study of Nigerian materials has shown it to be rich in protein and to have a high ash-content, but, however, with an oxalate-content high enough to be possibly lethal (Irvine, 1956).

Ecology and geographic distribution

The plant of *Talinum triangulare is* an exotic introduced from tropical America and the Caribbean, and now occurring in all parts of the West-Africa region from Senegal to southern Nigeria but not in the drier northern states. The plant is a common weed of rice-field in old Bendel state of Nigeria. It is frequently cultivated along the West African coast and even far inland (Okigbo, 1967).

Scientific Classification of T*alinum triangulare*

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Caryophyllidae*
Order	*Caryophyllales*
Family	*Portulacaceae* – Purslane family
Genus	*Talinum* Adans. – fameflower
Species	*Talinum triangulare* (Jacq.) Willd. – Ceylon spinach

Source: (USDA.gov) (Plant identifier, 2010)

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Figure 2.6: *T. triangulare* in its natural habitat: (Source: United States Department of Agriculture; USDA.gov – Plant identifier, 2010)

Table 2.3: Proximate, phytochemical and mineral compositions of Aqueous extract of *T. triangulare* leaves

Moisture Content (%)	Dry Matter (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate (%)
9.24	90.76	2.4	0.40	1.00	2.00	94.20

Alkaloids (%)	Saponin (%)	Flavonoids (%)	Anthocyanic (%)	HCN (mg/kg)	Sterols (%)	Tannin (%)
0.96	0.10	0.02	0.04	0.00	0.00	0.08

Ca mg/100g	Mg mg/100g	K mg/100g	Na mg/100g	P mg/100g	Fe mg/kg	Zn mg/kg	Pb mg/kg	Cu mg/kg	Cd mg/kg	Cr mg/kg
24	12.2	610	10	340	4.10	10	0.18	4.10	0.01	0.02

Source: Dike (2010)

Amino Acid Profile of *Talinun triangulare*.

Analysis of the amino Acid contents of *Talinum triangulare*

Types of amino acids analysis (g/16g N) of leaf meals.

Alanine	– 6.12	Cystine	– 1.30
Aspartic acid	- 7.01	Meth.±Cys.	- 3.40
Arginine	- 5.96	Leucine	- 9.02
Glycine	- 5.61	Serine	- 4.02
Glutamic acid	- 9.38	Threonine	- 4.10
Histidine	- 2.01	Phenyalanine	- 6.21
Isoleucine	- 5.62	Valine	- 6.10
Lysine	- 2.68	Tyrosine	- 4.71
Methionine	- 2.10	Tryptophan	- 1.82

Fasuyi (2006)

2.9.3 Principle of lyophylization

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 liquid goes directly to the gaseous state without passing through the liquid phase. 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. pre freezing, primary drying and secondary drying (Nireesha *et al.,* 2013)

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 makeup 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. 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 ((Nireesha *et al.*, 2013).

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. It is extremely important that the temperature at which a product is freeze dried is balanced between the temperature that maintains the frozen integrity of the product and the temperature that maximizes the vapour pressure of the product (Nireesha *et al.*, 2013).

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. 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 (Nireesha *et al.*, 2013)

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CHAPTER THREE

METHODOLOGY

This was an experimental study and this chapter focuses on the methods used in collection and extraction of plants (*vernonia amygdalina and talinum triangulare*) used, preparation of stock and working concentration of KCN and the plants extracts, administration of KCN and vegetable extracts, collection of blood and tissues samples and also the data management.

3.1 Aqueous extraction of *Vernonia amygdalina* and *Talinum triangulare*

Fresh plants (*Vernonia amygdalina and Talinum triangulare*) were purchased from a vegetable farm (Ajala Farm) in Iyana-Church Road, Ibadan Oyo State Nigeria. Identification and authentication of the plant was done at the University of Ibadan Herbarium. The leaves of both vegetables were picked separately and washed thoroughly with distilled water to remove dirt and contaminants, wet weight of 1kg each was taken after which 200 ml of distilled water added to blend into paste. The paste was poured into the cloth mesh and squeezed thoroughly to remove the extract. The volume of the extract was taken using a measuring cylinder. The extracts were stored and shipped at $35^{o}F$ – $46^{o}F$ ($+2^{o}C$ - $+8^{o}C$) temperature condition to the International Institute of Tropical Agriculture (IITA) for freeze drying.

3.1.1 Aqueous extraction of 'ewuro' *vernonia amygdalina*

Wet Weight of 'Ewuro' leaves = 1kg
Volume of distilled water used = 200 ml
Volume after aqueous extraction = 485 ml
Weight of lyophilizing tray = 222.00 g
Dry weight of 'Ewuro' extract = (Total weight of tray and extract – weight of tray) g
= (229.255 – 222.00) g
= 7.225 g

3.1.2 Aqueous extraction of 'gbure' *talinum triangulare*

Wet Weight of 'Gbure' leaves = 1kg

Volume of distilled water used = 200 ml

Volume after aqueous extraction = 650 ml

Weight of lyophilizing tray = 222.00 g

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

= (238.627 – 222.00) g

= 16.627g

3.2 Experimental animals

Thirty 7 weeks old Wistar rats were randomly assigned into Five (5) experimental groups and One (1) control group.

The animals were purchased from the animal house of the Post-graduate Institute for Medical Research and Training, Biode Building, University College Hospital to acclimatise for four weeks. They were obtained at 3 weeks old and were fed for four weeks on commercial rat pellets and water ad- libitum. They were kept in polyethylene cages whose dimensions are 30 cm by 15 cm by 25cm at room temperature. All the procedures were performed between 09:30 and 11:30 a.m.

3.3 Lyophilization (freeze drying)

300 ml of each vegetable extract was dispensed into the lyophilizer trays and was frozen for 5 hours. The frozen extract was then placed in the batch lyophilizer and freeze dried for 2 days. Powdery forms of the vegetable extracts were obtained at the end of the procedure.

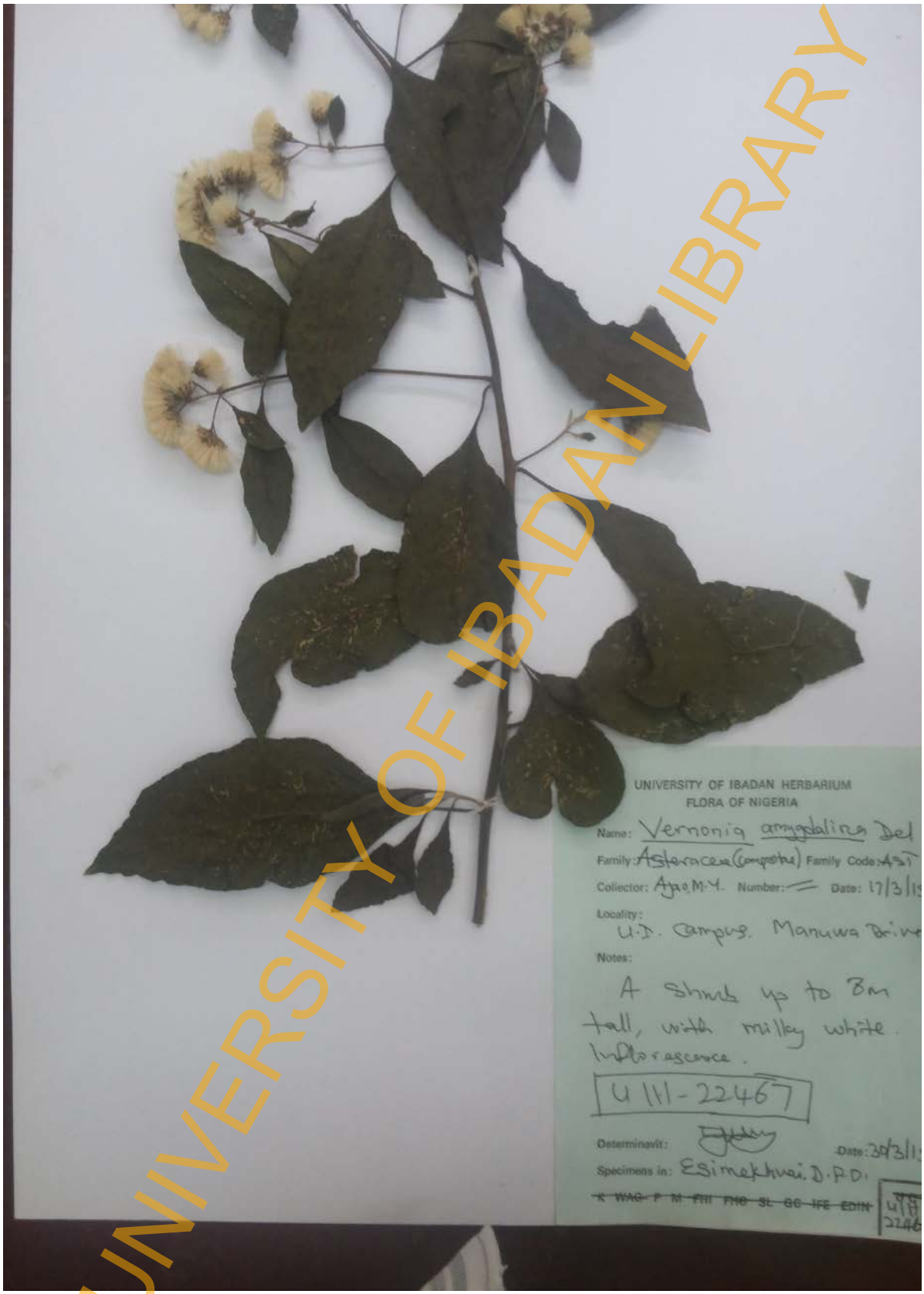

Plate 3.1: Voucher specimen of *V. amygdalina*

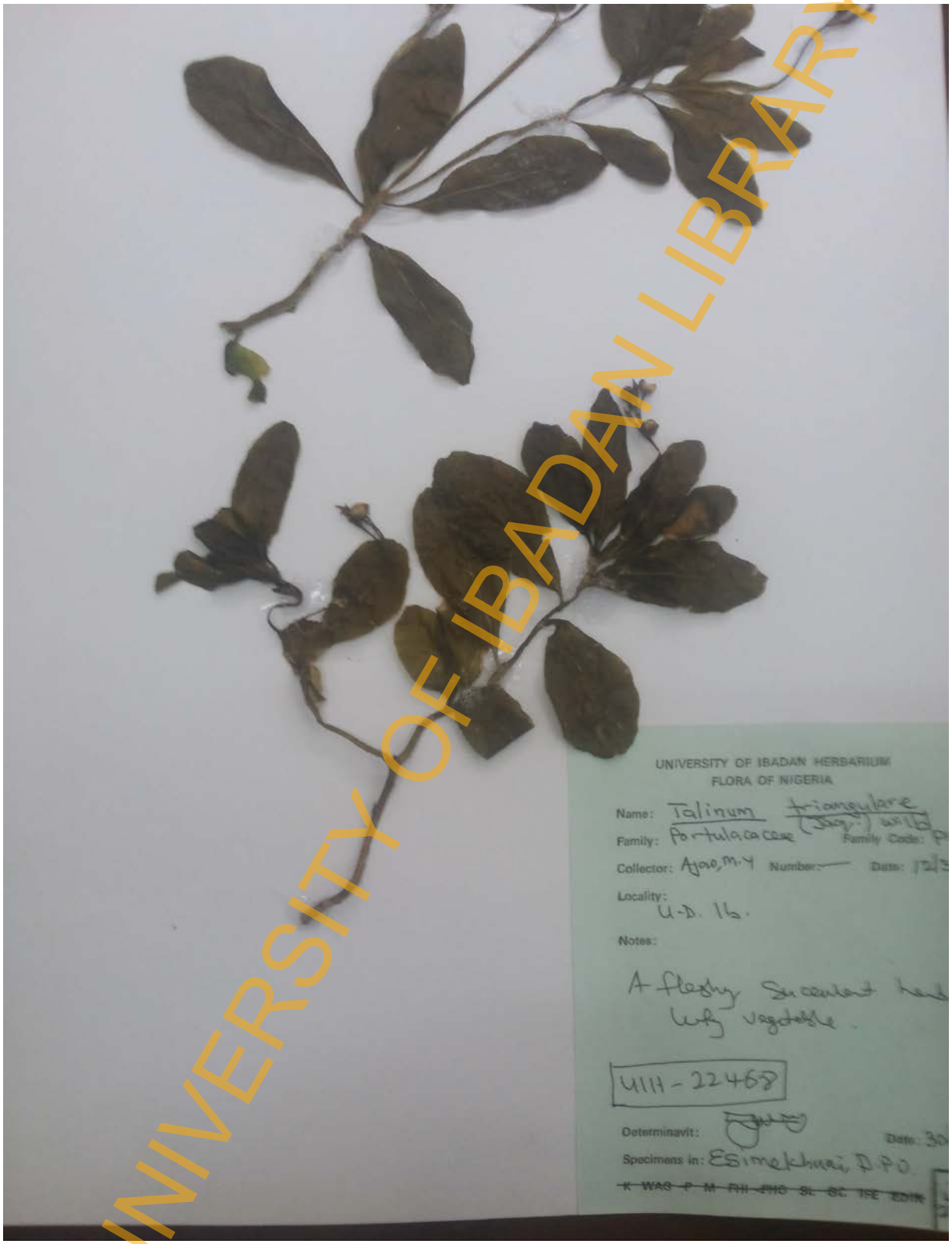

Plate 3.2: Voucher specimen of *T. triangulare*

3.4 Preparation of the stock and working concentration

Stock:

3mg of KCN was dissolved in 100 ml of distilled water to produce a concentration of 30 mg/L OR 30 mg/kg, and kept in a refrigerator.

3 mg of each vegetable extract was also dissolved in 100mls of distilled water to produce a concentration of 30 mg/L or 30 mg/kg, and kept in a refrigerator.

Working concentration:

1 ml of the KCN stock was dispensed in 9 ml of distilled water, shaken and covered with a foil paper and kept in the refrigerator. This is a 1 in 10 dilution (1:10) to produce a working concentration of 3 mg/L or 3 mg/kg.

1 ml each of the vegetable extract was dispensed in 9 ml 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 Procedure for administering KCN and the vegetable extracts

The animals were treated with the cyanide and or vegetable using an adjustable micropipette with plastic tips and a canular .The canular were labelled to prevent cross contamination. The weight of the rats was taken along with other physical observations before the KCN and the vegetable extracts were administered daily. Quantity of the KCN and the extracts administered were based on the weight of the rats. The ratio of the KCN 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 was mixed thoroughly before feeding it to rats. The small bottles, canular and tips were labelled as follows:

Group1 (n=5): Fed with equal volume of cyanide and distilled water based on the weight of animals (Cyanide only).

Group 2 (n=5): Fed with equal volume of cyanide and Vernonia amygdalina extract based on the weight of animals (Cyanide + VA)

Group 3 (n=5): Fed with equal volume of cyanide and *Talinum triangulare* extract based on the weight of animals (Cyanide + TA)

Group 4 (n=5): Fed with equal volume of Vernonia extract and distilled water based on the weight of animals (VA only)

Group 5 (n=5): Fed with equal volume of *Talinum triangulare* extract and distilled water based on the weight of animals (TA only)

Group 6 (n=5) : Fed with distilled water only

Assuming the weight of a rat in Group 2 i.e. (the CN+VA group) was taken as 190g, the micro pipette will be adjusted to 190µL and will be used to pick the cyanide and the extract into the small bottle, mixed together and make up to 1000µL before the canular was used to pick the mixture.

The animals were picked from the tail and the neck was gripped with the left hand and turned upwards with its limbs hanging up and the tail tucked between the hollow of the left hand, then a clear passage to the throat was sought before the mixture was administered. Before feeding, physical parameters i.e. agility, eye and fur colour, nose discharge and ocular and nasal lesion were observed and recorded for 2 weeks. Throughout the period of the study, a daily record of body weight, food and drinking water consumption was recorded

3.6 Collection of samples

3.6.1 Collection of blood samples

On day 14 from the first day of exposure, the rats were fasted for 24 hours before the termination of the treatments. On day 15, capillary tubes were used to collect blood samples from the rats while they were still alive using the ocular puncture method. The blood samples were placed inside Lithium heparinised bottles and were centrifuged at 1000 revolution/ minutes, after which the plasma collected by decanting into universal bottles before they were taken to Chemical Pathology where they were analysed for liver function enzymes (LFE); Alkaline Phosphatase (ALP), Aspartate Aminotransferases (AST), and Alanine Aminotransferases (ALT). Using capillary tubes, the Packed Cell Volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC), White Blood Cell (WBC), and Platelet Count of each rat was also estimated.

3.6.2 Collection of tissues

After the collection of blood samples, the animals were sacrificed. This was carried out by cervical dislocation method. The rats were held at the neck and tail and stretched until the spine dislocated from the neck. The carcass was cut open linea alba and the kidney, liver, brain, spleen and testes tissues were removed and placed in a sample bottle containing 10% Formalin for histopathological analysis.

3.7 Data management and statistical analysis

The daily record of body weight, food and water intake, haematological, biochemical and histological data were analyzed using descriptive statistics and results were expressed as mean and standard error of mean. In comparing the results of the groups, ANOVA and Student't-test were used and the difference was taken to be significant when P- value is <0.05.

CHAPTER FOUR

RESULTS

All experimental animals used survived to the end of the 14 days study. The results from the physical observations (body weight, ocular lesion and nasal discharge), haematology, biochemical analysis involving detecting levels of Alanine Amino Transferases (ALT) and Aspartate transaminase (AST) in serum and histopathology of sections of the brain, liver, kidney and the spleen are presented below.

4.1 Body weight

As shown in Table 4.1, the body weight change of animals in CN only, CN+VA, and CN+TA (43.4 ± 2.9, 21.4 ± 4.1 and 30.6 ± 5.3 g respectively) were comparable and not significantly ($p>0.05$) different from control (32.0 ± 7.5 g). VA only and TA only (3.8 ± 2.9 and 7.4 ± 4.2 g respectively) produced a significant ($p<0.05$) reduction in body weights of animals when compared with control (32.0 ± 7.5 g).

4.2 Food and water intake

4.2.1 Food intake

In respect of the food intake (Table 4. 1), there was a significant ($p<0.05$) increase in CN only (87.4 ± 6.4 g) treatment group compared with control (70.4 ± 5.2 g). However, CN+VA, CN+TA, VA only and TA only (71.7 ± 6.2, 71.7 ± 6.2, 79.0 ± 6.5 and 62.7± 5.7 respectively) were comparable and not significantly ($p>0.05$) different from control (70.4 ± 5.2 g).

4.2.2 Water intake

Considering the water intake as shown in Table 4.1, the value obtained for CN+VA (214.7 ± 31.2 g) produced a significant ($p<0.05$) increase in feeding compared with control (127.6 ± 24.2 g). Values obtained with the CN only, CN+TA, VE only and TA only (167.8 ± 23.8, 192.2 ± 24.5, 181.9 ± 27.8 and 100.2 ± 22.1 g respectively) were comparable and not significantly ($p>0.05$) different from control ((127.6 ± 24.2 g).

Table 4.1: Effects of treatments on body weight, food and water intake.

TREATMENTS	BODY WEIGHT CHANGE (g)	FOOD INTAKE (g)	WATER INTAKE (g)
CN Only	43.4 ± 2.9	87.4 ± 6.4$^{\beta}$	167.8 ± 23.8
CN + VA	21.4 ± 4.1	71.7 ± 6.2	214.7 ± 31.2$^{\gamma}$
CN +TA	30.6 ± 5.3	71.7 ± 6.2	192.2 ± 24.5
VE Only	3.8 ± 2.9$^{\alpha}$	79.0 ± 6.5	181.9 ± 27.8
TA Only	7.4 ± 4.2$^{\alpha}$	62.7± 5.7	100.2 ± 22.1
Control	32.0 ± 7.5	70.4 ± 5.2	127.6 ± 24.2

Values are mean ± SEM (n=5). $^{\alpha}p<0.05$ vs. control (One-way ANOVA followed by Tukey's multiple comparison tests).

Values are mean ± SEM (n=14). $^{\beta}p<0.05$ vs. control (One-way ANOVA followed by Tukey's multiple comparison tests).

Values are mean ± SEM (n=14). $^{\gamma}p<0.05$ vs. control (One-way ANOVA followed by Tukey's multiple comparison tests).

Table 4.2: Effects of treatments on organs (Organs/body weight ratio)

TREATMENTS	liver wt (mg)	kidney wt (mg)	spleen wt (mg)	brain wt (mg)
CN Only	35.4 ± 1.1	8.0 ± 0.9	2.9 ± 0.4	24.6 ± 6.1
CN + VA	30.5 ± 9.0	7.9 ± 0.4	2.9 ± 0.3	8.7 ± 0.9
CN + TA	32.5 ± 0.9	7.6 ± 0.1	2.3 ± 0.3	7.6 ± 1.7
VE Only	41.5 ± 3.2	8.8 ± 0.4	2.7 ± 0.5	$10.9 \pm 0.6^{\alpha}$
TA Only	31.9 ± 1.1	8.9 ± 0.6	2.3 ± 0.2	$11.3 \pm 0.3^{\alpha}$
Control	33.9 ± 4.5	7.6 ± 0.8	3.4± 0.5	09.2 ± 0.4

Values are mean ± SEM (n=5). $^{\alpha}p<0.05$ vs. Control (One-way ANOVA followed by Tukey's multiple comparison test).

Table 4.3: Effects of treatments on hematology parameters

HAEMATOLOGY					
GROUPS	PCV %	HB mg/dL	RBC mm^3	WBC X 10^3 mm^3	PLATELET X 10^4 mm^3
CN Only	35.4 ± 0.6	11.1 ± 0.2	6.0 ± 0.6	7700 ± 1218	111000 ± 1617
CN + VE	38.4 ± 1.6	12.1 ± 0.5	6.2 ± 0.6	8690 ± 1202	95800 ± 3747
CN + TA	35.6 ± 0.9	10.8 ± 0.2	6.1 ± 0.7	10000 ± 1450	83800 ± 13890
VE Only	45.2 ± 3.3[a]	14.0 ± 2.3[α]	7.4 ± 0.9[β]	10890 ± 1716	125000 ± 14930
TA Only	35.8 ± 1.0	11.0 ± 0.3	5.9 ± 0.6	9920 ± 1598	130200 ± 14530
Control	37.0 ± 4.4	12.4 ± 0.5	6.8 ± 0.0	8500 ± 1197	85800 ± 14770

Values are mean ± SEM (n=5). [a]$p<0.05$ vs. control. (One-way ANOVA followed by Tukey's multiple comparison test).

Values are mean ± SEM (n=5). [α]$p<0.05$ vs. control. (One-way ANOVA followed by Tukey's multiple comparison test).

Values are mean ± SEM (n=5). [β]$p<0.05$ vs. control. (One-way ANOVA followed by Tukey's multiple comparison test).

Table 4.4: Effects of treatments on hematology parameters

Groups	LYM %	NEU %	MON %	EOS %
CN Only	63.2 ± 11.8	34.2 ± 11.7	0.8 ± 0.5	1.8 ± 0.5
CN + VA	74.4 ± 14.0	27.0 ± 7.9	0.8 ± 0.8	1.6 ± 1.1
CN + TA	63.6 ± 8.5	32.6 ± 8.7	1.2 ± 0.8	2.6 ± 2.2
VA Only	71.2 ± 7.9	26.4 ± 7.9	1.4 ± 0.9	1.0 ± 0.7
TA Only	68 ± 4.8	30.6 ± 3.5[a]	0.8 ± 0.8	0.8 ± 0.8
Control	70.8 ± 2.5	26.4 ± 1.7	1.4 ± 0.9	1.4 ± 1.1

Values are mean ± SEM (n=5). [a]$p<0.05$ vs. control. (One-way ANOVA followed by Tukey's multiple comparison test).

4.3 Organs - body weight ratio

In Table 4.2, there was generally no significant difference ($p>0.05$) in the values obtained in the experimental groups (CN only, CN+VA, CN+TA, VA only, and TA only) on liver, kidney and the spleen when compared with control. However, VA only and TA only (10.9 ± 0.6 and 11.3 ± 0.3 g respectively produced a significant ($p<0.05$) weight increase in brain compared with control (9.2 ± 0.4 g).

4.4 Haematological analysis

Table 4.3 showing the mean values and the standard error of the means for packed cell volume (PCV), haemoglobin count (Hb), red blood cell count (Rbc), white blood cell count (Wbc) and platelets (Pl).

4.4.1 Packed cell volume

In Table 4.3, packed cell volume (PCV) showed a siginificant ($p<0.05$) increase in the mean values of VA only (45.2 ± 3.3) when compared with control (37.0 ± 4.4). However, a non-significant($p>0.05$) but comparable values in CN only, CN+VA, CN+TA and TA only (35.4 ± 0.6, 38.4 ± 1.6, 35.6 ± 0.9 and 35.8 ± 1.0 respectively) were observed when compared with control (37.0 ± 4.4).

4.4.2 Haemoglobin

As shown in Table 4.3, in respect of Hb, a siginificant ($p<0.05$) increase in the mean values of VA only (14.0 ± 2.3) when compared with control (12.4 ± 0.5). However, a non-significant ($p>0.05$) but comparable values in CN only, CN+VA, CN+TA and TA only (11.1 ± 0.2, 12.1 ± 0.5, 10.8 ± 0.2 and 11.0 ± 0.3 respectively) were observed when compared with control (37.0 ± 4.4).

4.4.3 Red Blood Cell

In Table 4.3, considering Rbc, a siginificant ($p<0.05$) increase in the mean values of AE only (7.4 ± 0.9) when compared with control (6.8 ± 0.0). However, a non-significant($p>0.05$) but comparable values in CN only, CN+VA, CN+TA and TA only

61.0 ± 0.6, 6.2 ± 0.6, 6.1 ± 0.7 and 5.9 ± 0.6 respectively) were observed when compared with control (6.8 ± 0.6).

4.4.4 White blood cell count

In Table 4.3, the mean values of white blood cell count showed no significant difference ($p>0.05$) when animals in treatment groups (CN only, CN+VA, CN+TA, VA only and TA only (7700 ± 1218, 8690 ± 1202, 10000 ± 1450, and 10890 ± 1716 respectively) were compared with control (8500 ± 1197). Moreover, there was no significant ($p>0.05$) difference in the white blood cell differentials (lymphocytes, neutrophils, monocytes and eosinophils) with treatments; CN only, CN+VA, CN+TA, VA only respectively. However a significant ($p<0.05$) increase was produced when mean values of TA only (9920 ± 1598) compared with control (8500 ± 1197) in neutrophils as shown in Table 4.4.

4.5 Platelets count

There was generally no significant ($p>0.05$) difference in the mean values for platelets count obtained from animals treated with CN only, CN+VE, CN+TA, VA only and TA only respectively (111000 ± 1617, 95800 ± 3747, 83800 ± 13890, 125000 ± 14930 and 130200 ± 14530 respectively) when compared with control (85800 ± 14770) as shown in Table 4.3 .

Table 4.5: Effects of treatments on biochemical parameters

Groups	Total protein mg/dL	Albumin mg/dL	Globulin mg/dL	AST mg/dL	ALT mg/dL	Bilirubin mg/dL	Creatinine mg/dL
CYN ONLY	4.4 ± 0.9	1.9 ± 0.2	2.5 ± 0. 8	29.0 ± 8.9	18.0 ± 3.5	10.0 ± 2.0	0.5 ± 0.1
CYN + VE	5.1 ± 0.2	2.3 ± 0.3	2.8 ± 0.4	33.0 ± 5.2	18.0 ± 7.0	6.7 ± 1.5$^{\beta}$	1.0 ± 0.3
CYN + TA	4.5 ± 0.3^{a}	2.8 ± 0.1	1.7 ± 0.4$^{\alpha}$	19.0 ± 7.6	22.0 ± 8.6	9.0 ± 2.6	0.9 ± 0.3
VE ONLY	5.7 ± 0.6	2.9 ± 0.6	2.9 ± 0.1	25.0 ± 13.0	19.0 ± 2.1	9.7 ± 1.5	0.7 ± 0.3
TA ONLY	5.9 ± 0.4^{a}	2.7 ± 0.6	3.1 ± 0.4	16.0 ± 7.2	17.0 ± 4.7	10.0 ± 2.1	0.6 ± 0.5
CONTROL	5.2 ± 0.2	2.3 ± 0.5	2.9 ± 0.4	21.0 ± 6.1	21.0 ± 7.1	11.0 ± 1.0	0.8 ± 0.3

Values are mean ± SEM (n=5). $^{a}p>0.05$ vs control (One-way ANOVA followed by Tukey's multiple comparison test).

Values are mean ± SEM (n=5). $^{\alpha}p>0.05$ vs control (One-way ANOVA followed by Tukey's multiple comparison test).

Values are mean ± SEM (n=5). $^{\beta}p>0.05$ vs control (One-way ANOVA followed by Tukey's multiple comparison test).

4.5 Biochemical analysis

4.5.1 Total protein (TP)

In Table 4.5, there was a significant ($p<0.05$) difference in the mean values of total protein when treatment groups; CN+TA and TA only (4.5 ± 0.3 and 5.9 ± 0.4) compared with (5.2 ± 0.2). However, a non significant ($p>0.05$) biological difference in the mean values total total protein with treatments; CN only, CN+VA, VA only (4.4 ± 0.9, 5.1 ± 0.2, , 5.7 ± 0.4 respectively) compared with control (5.2 ± 0.2).

4.5.2 Albumin

As shown in Table 4.5, mean values for albumin in treatment groups; CN only, CN+VA, CN+TA, VA only and TA only (1.9 ± 0.2, 2.3 ± 0.3, 2.8 ± 0.1, 2.9 ± 0.6 and 2.7 ± 0.6 respectively) are comparable but not significantly($p>0.05$) different from control (2.3 ± 0.5).

4.5.3 Globulin

In Table 4.5, a significant ($p<0.05$) decrease in the mean value of globulin was produced with treatment CN+TA (1.7 ± 0.4) when compared with control (2.9 ± 0.4). Moreover, a non significant ($p>0.05$) difference was observed with mean values of globulin in the experimental groups; CN only, CN+VA, VA only and TA only (2.5 ± 0.8, 2.8 ± 0.4, 2.9 ± 0.1 and 3.1 ± 0.4 respectively) when compared with control (2.9 ± 0.4).

4.5.4 Aspartate transaminase (AST) and Alanine amino transaminase (ALT)

As shown in Table 4.5, mean values for AST in all experimental groups; CN only, CN+VA, CN+TA, VA only and TA only (29.0 ± 8.9, 33.0 ± 5.2, 19.0 ± 7.6, 25.0 ± 13.0 and 16.0 ± 7.2 respectively) are comparable and not significantly ($p>0.05$) different from control (21.0 ± 6.1). However, there was no significant difference ($p>0.05$) in mean values of ALT when the experimental groups CN only, CN+VA, CN+TA, VA only and TA only (18.0 ± 3.5, 18.0 ± 7.0, 22.0 ± 8.6, 19.0 ± 2.1 and 17.0 ± 4.7 respectively) were compared to control (21.0 ± 7.1).

4.5.5 Bilirubin and Creatinine

In Table 4.5, a significant ($p<0.05$) decrease was produced in the mean values for bilirubin (6.7 ± 1.5) compared with control (11.0 ± 1.0) when animals in group 2 were treated with CN+VA. Mean values for creatinine were not significantly ($p>0.05$) different when the experimental groups compared with control.

4.6 Histopathological analysis

A random selection of three animals (rats) from each experimental group for histopathological analysis was carried out. The results of the analysis of the liver, kidney, spleen and the brain are presented in Table 4.6 and Plates 4.1 to 4.14

4.7 Ocular lesion and nasal discharge

There were no visible sign of ocular lesion in animals of group one to six. No nasal discharge was observed in animals of group two, three, and four but slimy nasal discharge was found in 20 per cent of rats in group one and 10 per cent of rats in group five only as shown in Figure 4.1

Table 4.6: Histopathological analysis of the organs

GROUP	LIVER	KIDNEY	SPLEEN	BRAIN
1a	Multifocal fatty degeneration	Mild congestion	No visible lesion	No visible lesion
1c	Fatty degeneration	Mild necrosis	Lymphoid depletion	Multifocal area spongiosis
1d	Portal lymphocytic degeneration	Glomerular cast	No visible lesion	No visible lesion
2a	Slight fatty degeneration	No visible lesion	No visible lesion	No visible lesion
2c	No visible lesion	No visible lesion	Lymphoid depletion	No visible lesion
2e	No visible lesion	No visible lesion	No visible lesion	No visible lesion
3a	No visible lesion	No visible lesion	No visible lesion	No visible lesion
3b	Hepatic necrosis	Mild congestion	No visible lesion	No visible lesion
3d	No visible lesion	No visible lesion	No visible lesion	No visible lesion
4a	No visible lesion	No visible lesion	No visible lesion	No visible lesion
4b	Hepatic necrosis	Mild congestion	No visible lesion	No visible lesion
4d	No visible lesion	No visible lesion	No visible lesion	No visible lesion
5a	No visible lesion	No visible lesion	No visible lesion	No visible lesion
5b	No visible lesion	No visible lesion	No visible lesion	No visible lesion
5e	Sinusoidal dilatation	No visible lesion	No visible lesion	No visible lesion
6b	No visible lesion	No visible lesion	No visible lesion	No visible lesion
6d	No visible lesion	No visible lesion	No visible lesion	No visible lesion
6e	No visible lesion	No visible lesion	No visible lesion	No visible lesion

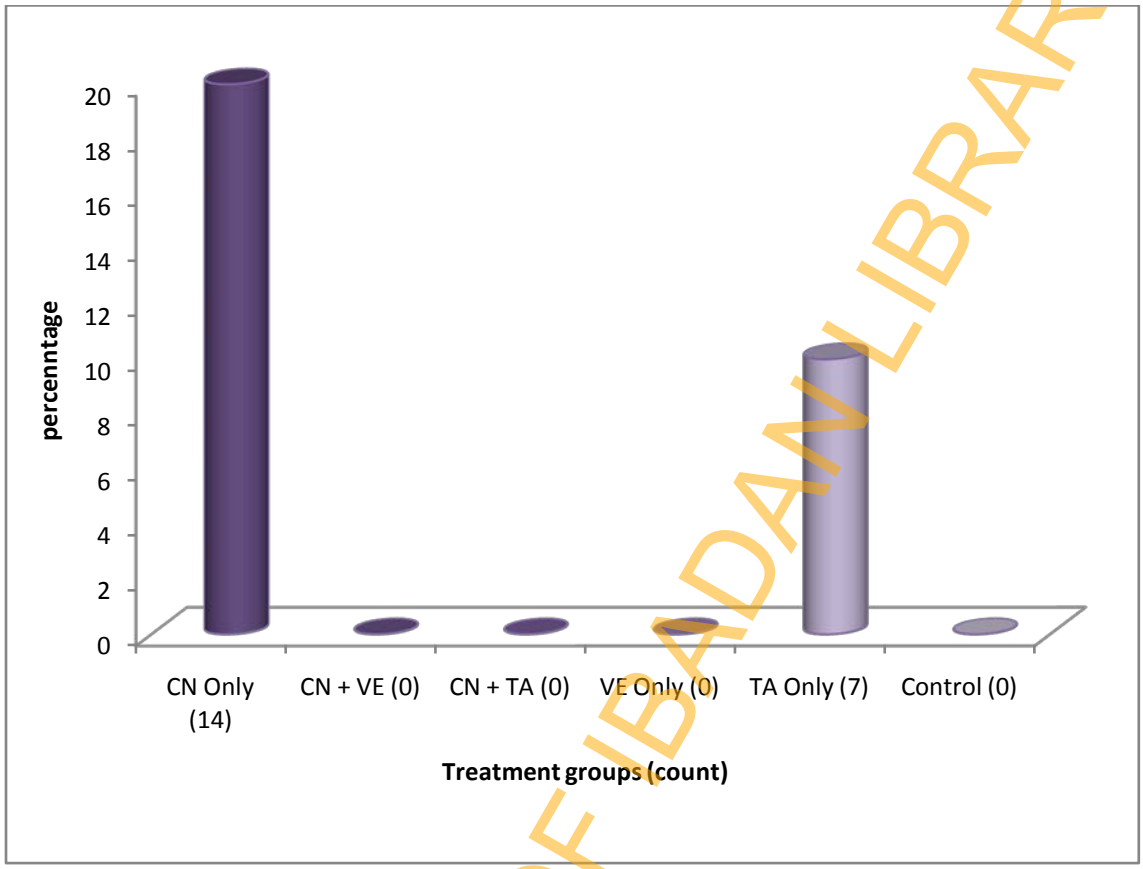

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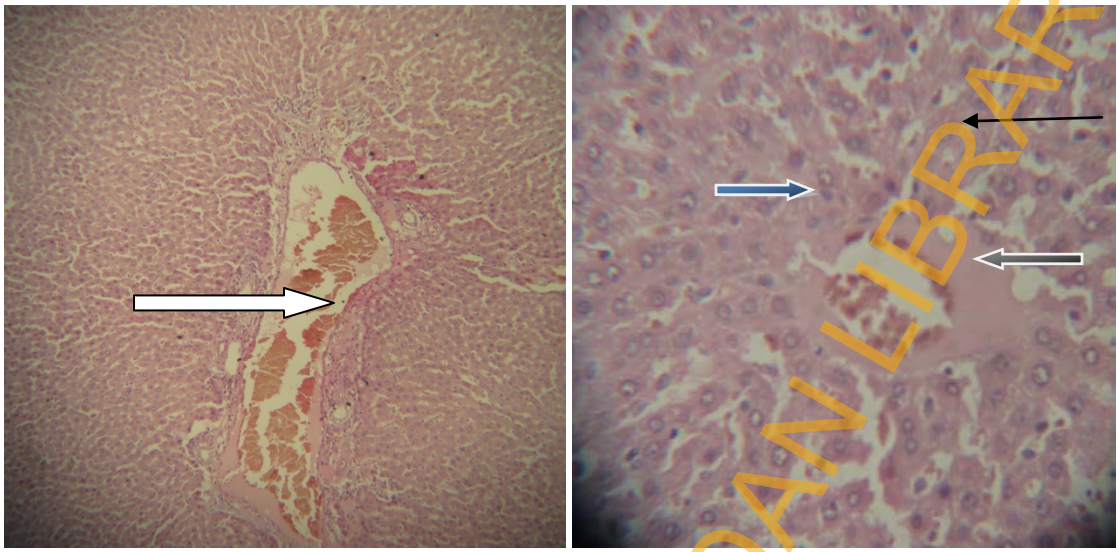
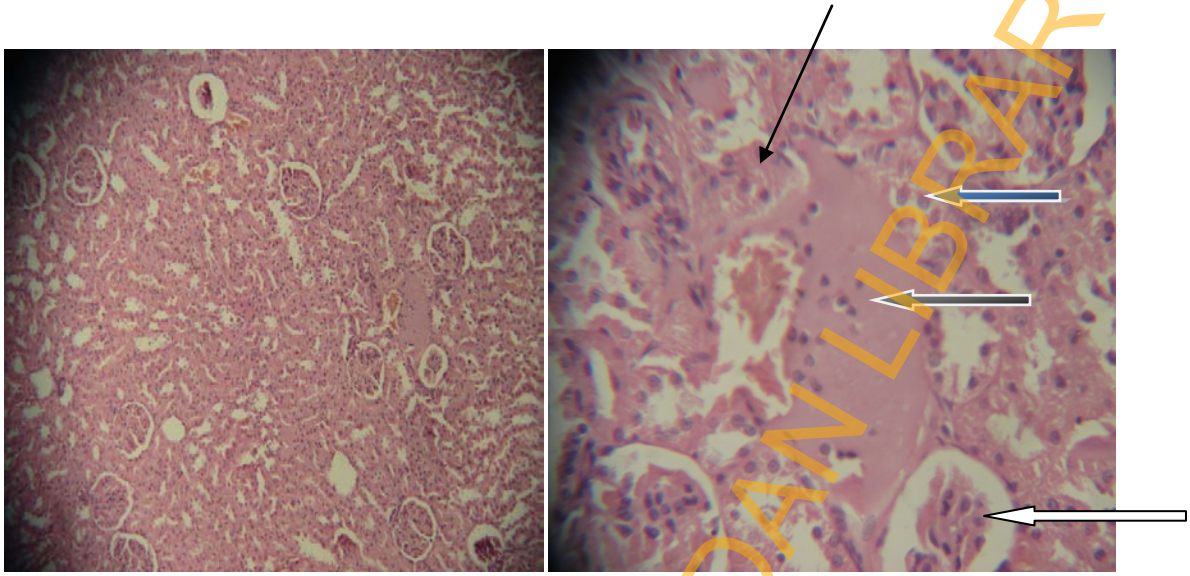
Figure 4.1: Percentage count of nasal discharge (n=70)



X100 **X400**

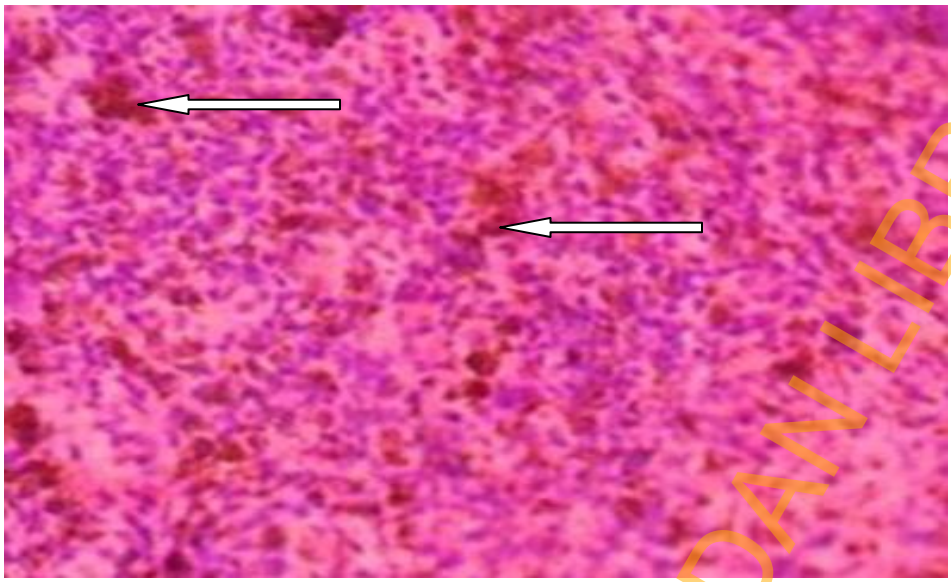
Plate 4.1: Photomicrography of liver section for group 1 (CN only)

Showing mild congested vessle with multifocal fatty degeneration (black arrow), the sinusoids are infiltrated by inflammatory cells (slender arrow), the lymphocytes and polymorphornuclear cells. The portal tract (white arrow) appear mildly infiltrated. The hepatocytes are normal (blue arrow).



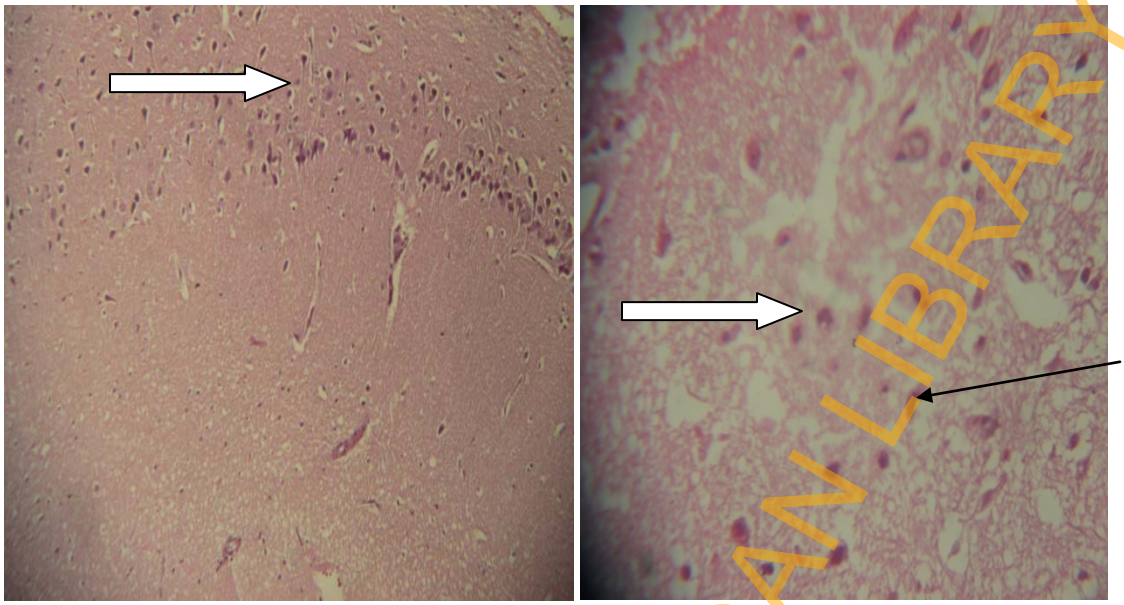
X100 X400

Plate 4.2: Photomicrography of kidney sectoin for group 1 (CN only)
Showing poor archutecture, the glomeruli appear normal (white arrow), the renal tubules shows mild tubular necrosis (blue arrow). There is focal area of accumulate fluid that appear eosinophilic (black arrow), the interstitial space shows mild infiltration (slender arrow).



X100
Plate 4.3: Photomicrography of spleen section for control 1 (CN only)

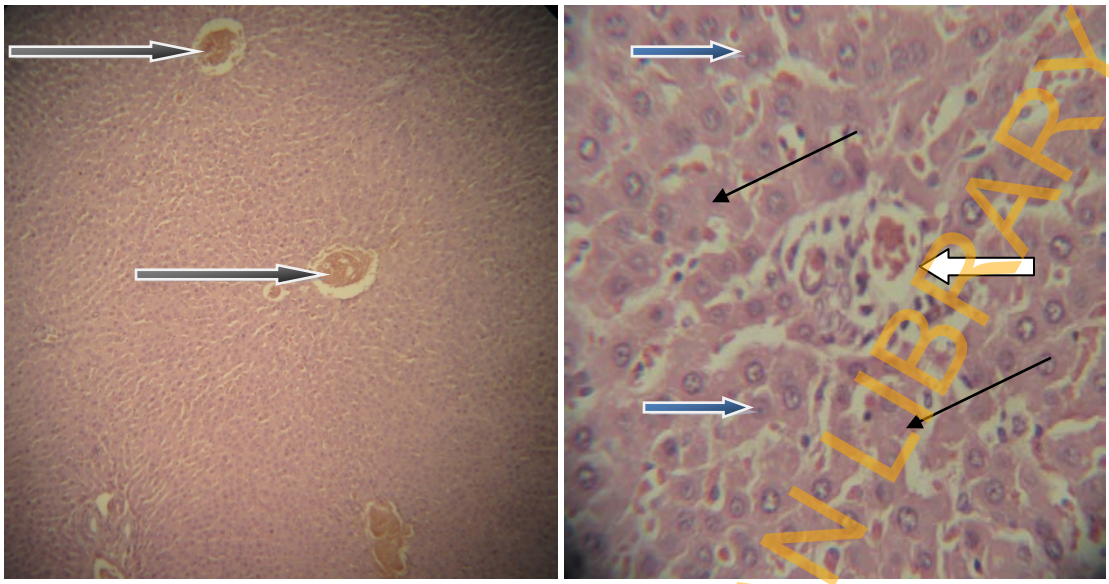
Showing lymphoid depletion, necrosis of the white pulp (white arrow) characterized by apoptosis of lymphocytes.



X100 x400

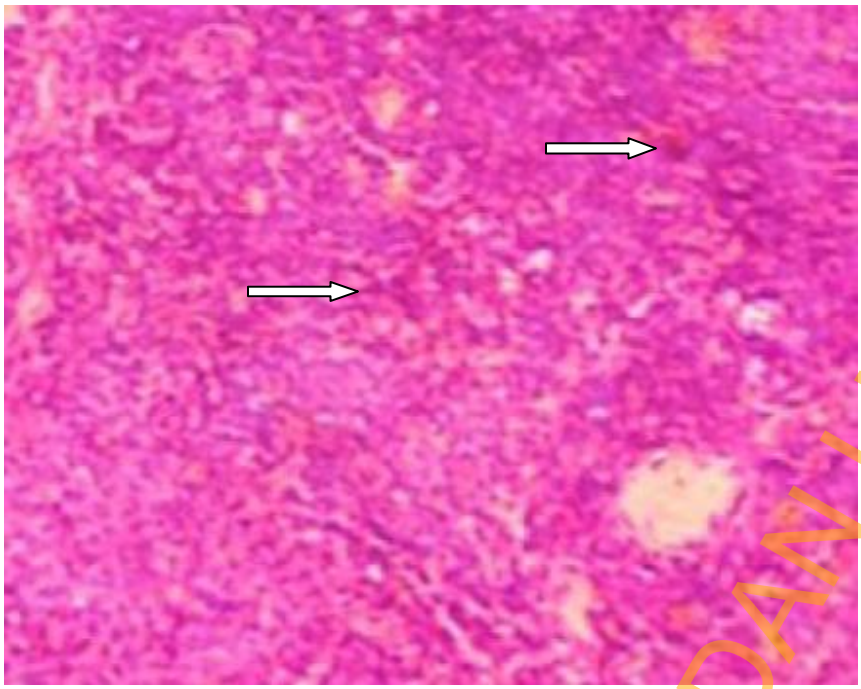
Plate 4.4: Photomicrography of brain section for control 1 (CN only)

Showing abnormal cerebellar cortex with moderate necrosis (white arrow) within the stroma and several observable necrotized neuronal cells seen (slender arrow)



X100 X400

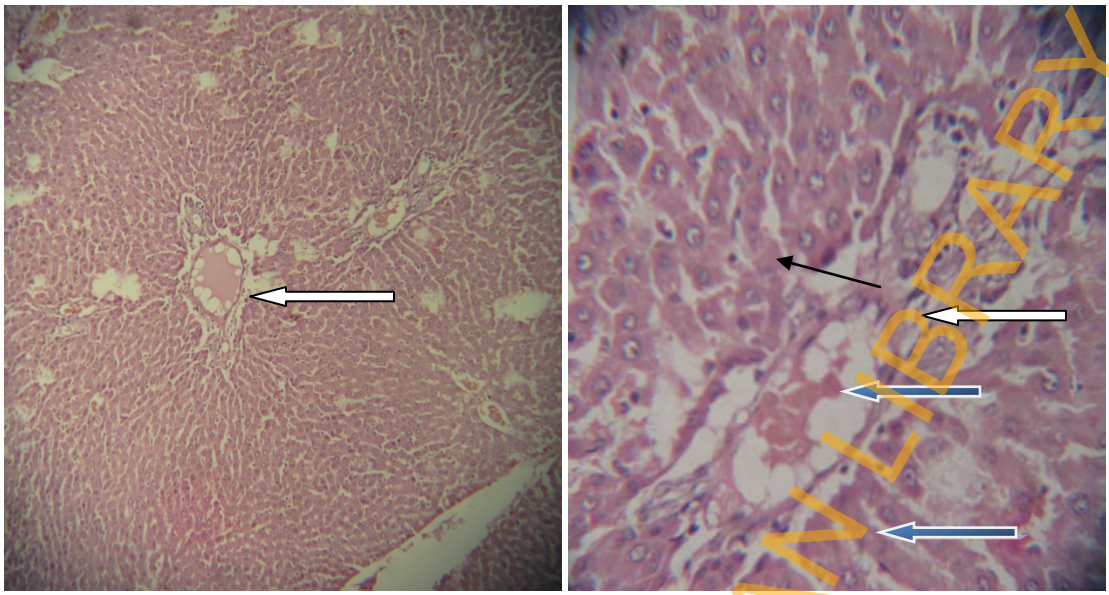
Plate 4.5: Photomicrography of liver section for group 2 (CN+VE)
Showing mildly congested vessle (black arrow), the sinusoids are moderately dilatated (slender arrow). The portal tract (white arrow) appear mildly infiltrated and the hepatocytes appear normal



X100

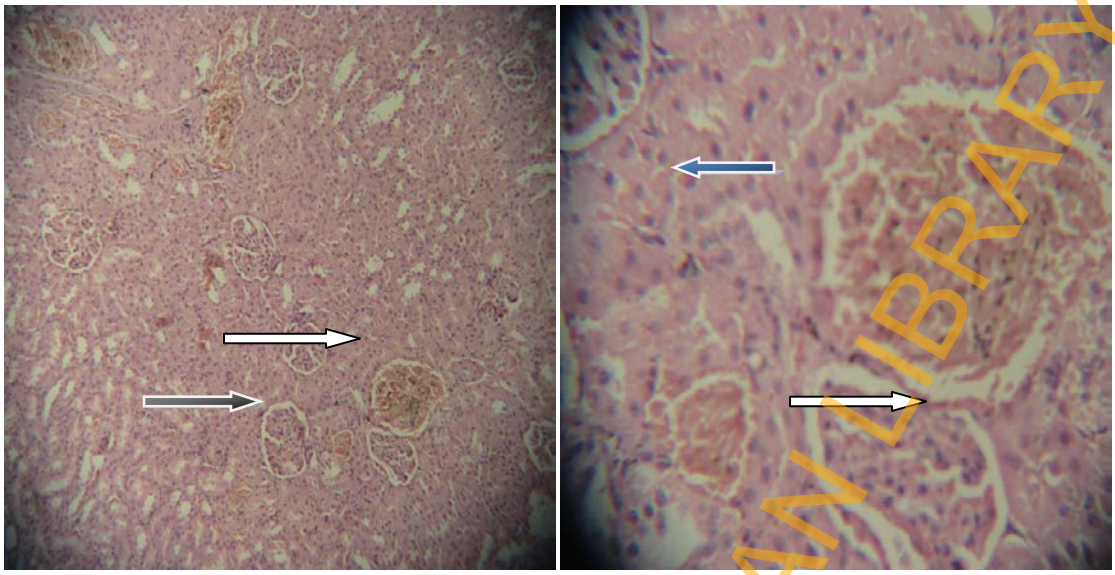
Plate 4.6: Photomicrography of spleen section for group 2 (CN + VE)

Showing slight lymphoid depletion of the white pulp (white arrow).



X100 X400

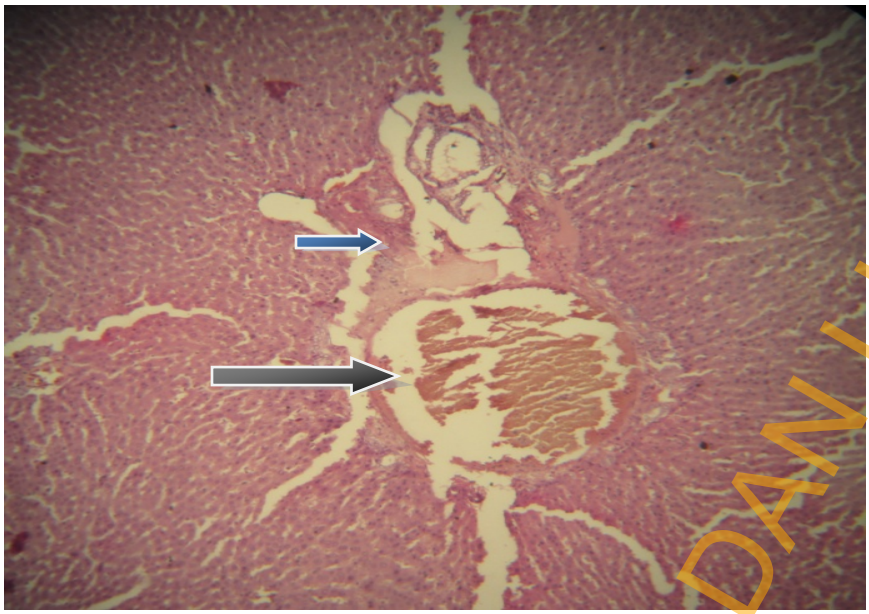
Plate 4.7: Photomicrography of liver section for group 3 (CN+ TA)
Showing mild congested portal vein (black arrow), the central vessles are not congested (white arrow), the sinusoids appear normal and the hepatocytes appear normal (blue arrow)



X100 X400

Plate 4.8: Photomicrography of kidney section for group 3 (CN+ TA)

Showing poor architecture, the glomeruli appear normal (white arrow), the renal tubules shows moderate tubular necrosis (blue arrow). There is moderate vascular congestion seen (black arrow), the interstitial space shows no infiltration (blue arrow).



X400

Plate 4.9: Photomicrography of liver section for group 4 (VE only)
Showing mild congested vessle (black arrow) with the portal tract (white arrow) also appear mildly infiltrated. The hepatocytes appear normal.

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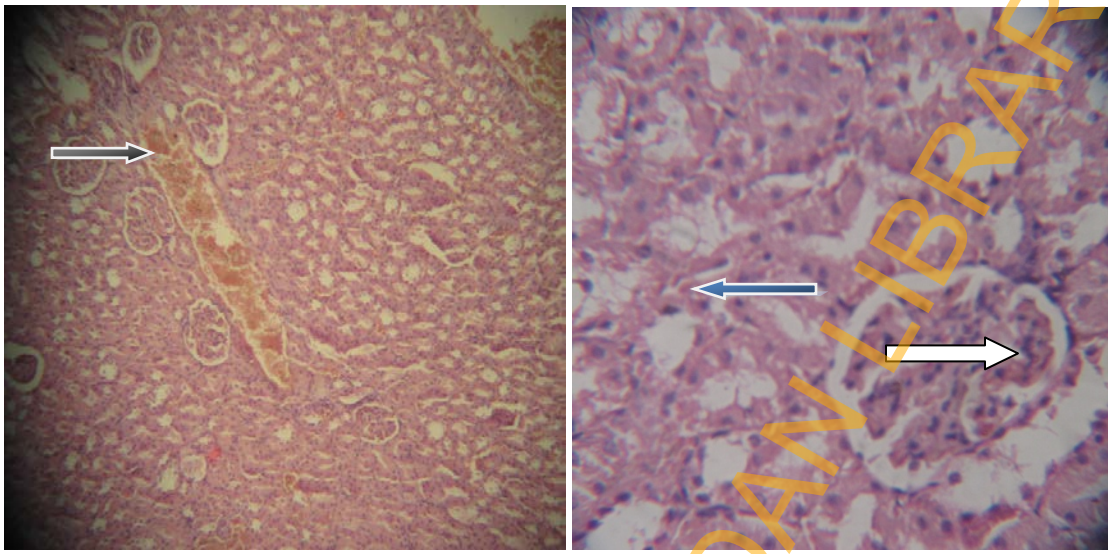
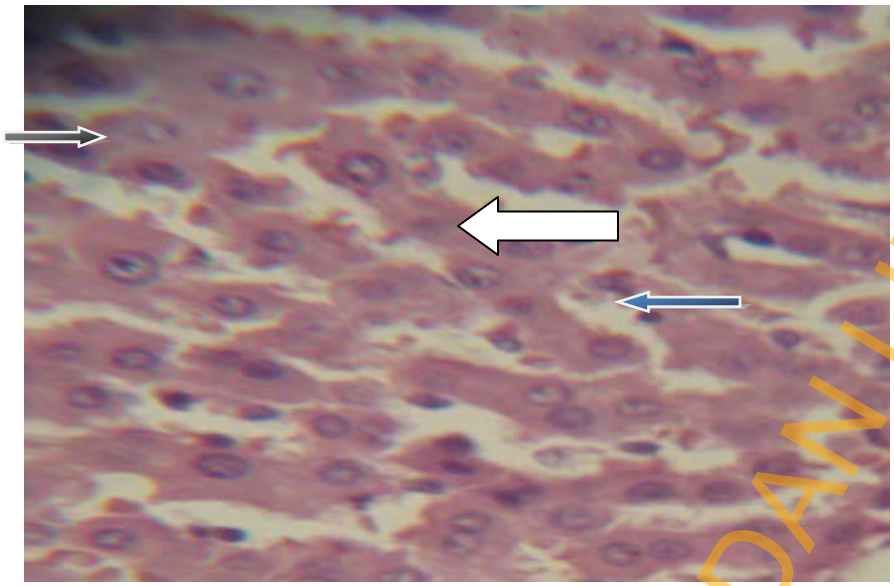


X100 X400

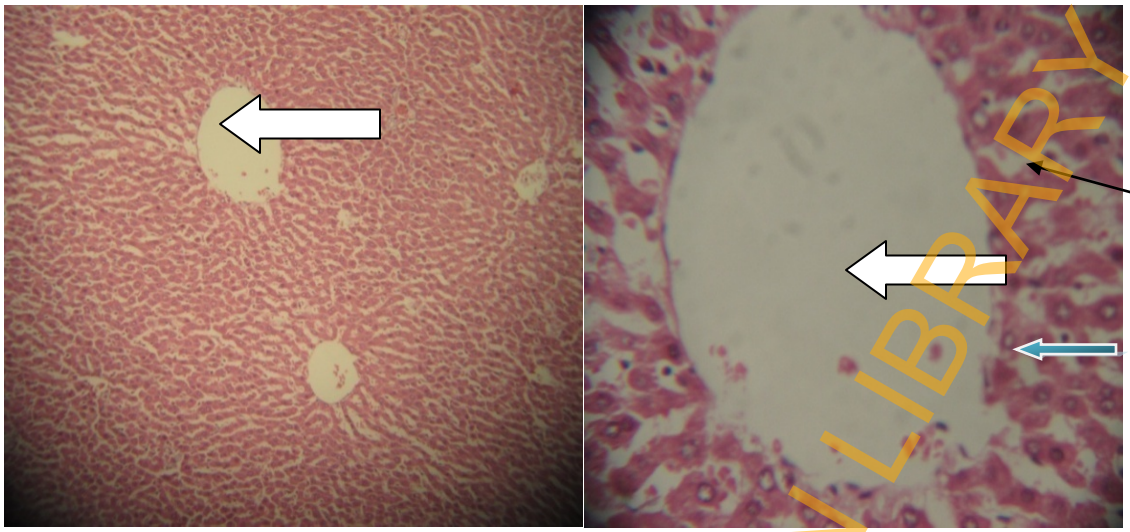
Plate 4.10: Photomicrography of kidney section for group 4 (VE only)
Showing poor architecture, the glomeruli appear normal (white arrow), the renal tubules shows mild tubular necrosis (blue arrow). There is vasular congestion (black arrow) and the interstitial space shows mild infiltration.



X400

Plate 4.11: Photomicrography of liver section for group 5 (TA only)

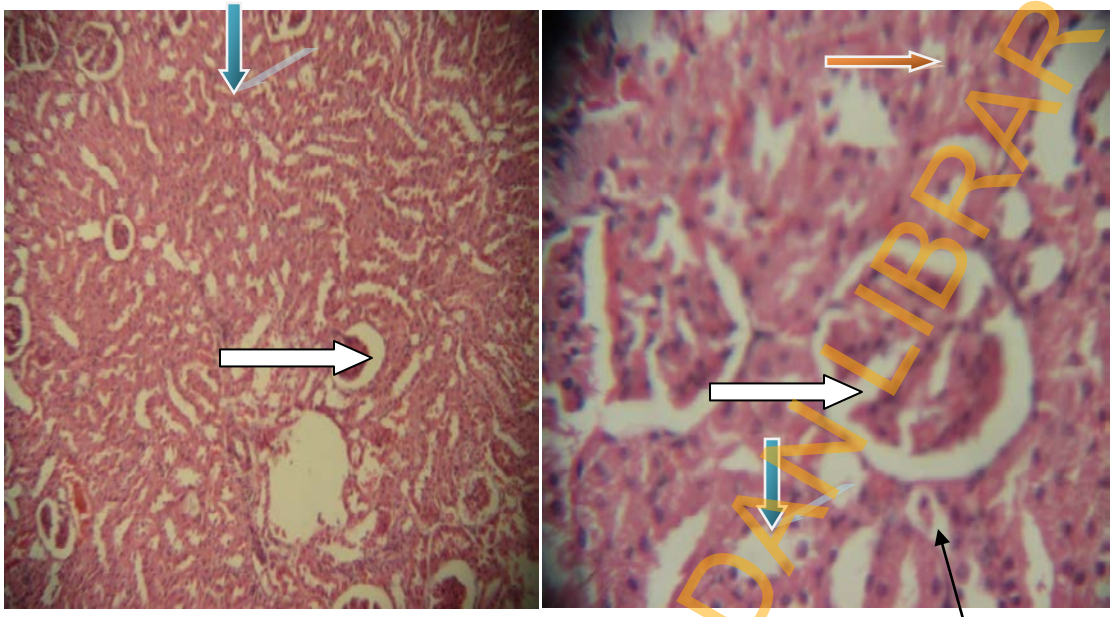
Showing normal architecture, the central vessle (blue arrow) is not congested, no vascular (black) congestion, the sinusoids appear midly infiltrated (white arrow). No pathological lesion seen.



X100 X400

Plate 4.12: Photomicrography of liver section for group 6 (control)

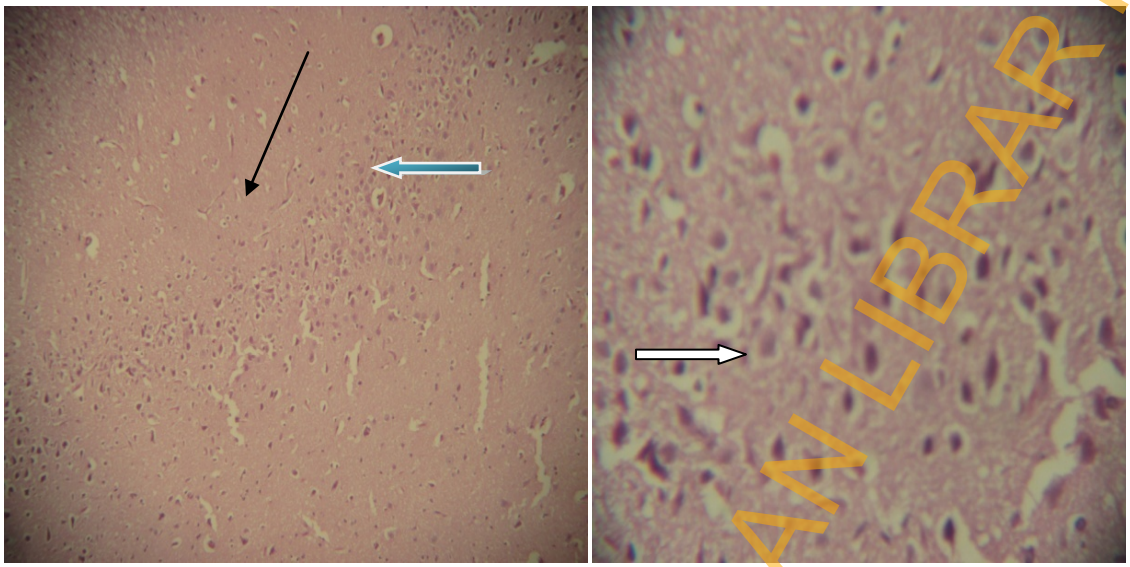
Showing normal architecture, the central vessle is not congested (white arrow), no vascular congestion, the sinusoids are normal without infiltration (slender arrow). The morphology of the hepatocytes appear normal (blue arrow). No pathological lesion seen.



X100 **X400**

Plate 4.13: Photomicrography of kidney section for control group

Showing normal architecture, the glomeruli (white arrow) is normal, the renal tubules containing distal convoluted tubules (red arrow) and proximal convoluted tubules (blue arrow) appear normal, the interstitial space appear normal (slender arrow). No pathological leison seen.



X100 X400

Plate 4.14: Photomicrography of brain section for control group
Showing normal cerebellum, the cortex (black arrow) appear normal, the hypocellular and granular layer (blue arrow) appear normal, the purkinje cells (white arrow) and other neuronal cells (slender arrow) appear normal, No necrosis.

CHAPTER FIVE

DISCUSSION

This chapter discusses the results obtained from the body weight changes, organs-body weight index in animals of various experimental groups and the control. Furthermore, results from the haematological, biochemical and histopathological indices were also discussed.

5.1 Body weight change of the treatment groups

Weight loss or gain largely depend on the nutritional status of the animals throughout the period of study which may result from decrease or increase in appetite for food and water. In this study, no significant body weight change was observed in animals fed with sub-acute dose of cyanide only, cyanide with *Vernonia amygdalina* and cyanide with *Talinum triangulare*. Moreover, a significant increase in food and water consumption were observed in animals of group one and two respectively. According to (Howard and Hanzal, 1955), in a 2-year feed study in which rats were administered feed containing hydrogen cyanide at concentrations up to 300ppm, there were no decreases in body weight gain. Also, administration of potassium cyanide at concentrations of up to 300ppm orally to rats and mice for 6 weeks resulted in no significant adverse effects on body weight in a study conducted by Philbrick *et al*., (1979). This study is in agreement with findings of these previous studies cited.

5.2 Organ-body weight index of the treatment groups

The organs weight ratio in all treatments when liver, kidney, spleen and the brain were considered in this experiment showed no significant difference when compared to control group. Although, *Vernonia amygdalina* extract only and *Talinum triangulare* extract only produced significant changes in organ-body weight index of the brain. Previous studies have shown that sub-acute exposure of potassium cyanide in male rats following oral administration of 7.0 mg/kg for 21 days did not produce any significant change in body weight and organ-body index of the animals according to Tulsawani *et*

al., 2005. Also, there were no effect on the liver weight occurred when potassium cyanide was administered in the diet at dose of 200mg/kg diet (Palmer and Oslon, 1979).

5.3 Ocular lesion and nasal discharge of the treatment groups

In cyanide treated animals, ocular lesion and blindness result from persistent anoxia in the brain. Visual and other neurological disturbances attributed to cyanide generally occur when exposed to relatively high levels of cyanide or cyanogenic compounds.

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). Numerous studies have implicated cyanide as the etiologic agent in human neuropathies, including Nigerian nutritional neuropathy, tobacco amblyopia, and Leber's optical atrophy (Towill *et.al.*, 1978).

There were no visible signs of ocular lesion in all experimental animals treated during this study; these findings could be as a result of short term exposure of the animals to sub-acute concentrations of cyanide. No nasal discharge was observed in animals of group two, three, and four but slimy nasal discharge was found in 20 per cent of rats in group one and 10 per cent of rats in group five only.

5.4 Haematology analysis of the treatment groups

In this study, sub-acute exposure of rats to cyanide did not produce any significant haematological changes but an increase in packed cell volume, haemoglobin counts and red blood cells were observed with the plant extracts of *Vernonia amygdalina* only, while extracts of *Talinum triangulare* only also caused an increase in neutrophils parameters. 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 21 days, various haematological and biochemical indices were determined after 7 days of treatment. Sub-acute exposure of KCN did not produce any significant change in haematology and the levels of blood urea, creatinine, aspartate aminotransferase, triiodothyronine (T3) and tetraiodothyronine (T4).

5.5 Biochemical analysis of the treatment groups

Liver function enzymes; aspartate transaminase (AST), alanine amino tranaminase (ALT) and alkaline phosphatase (ALP) are useful biomarkers in the plasma. AST 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 (Anonymous, 1976).

In this study, adult Wistar rats sub-acutely exposed to cyanide, cyanide with vegetable extracts of *Vernonia amygdalina* and *Talinum triangulare* showed a comparable but not significant changes in liver function enzymes; aspartate transaminase (AST), alanine amino transaminase (ALT). A significant reduction and increase in total protein respectively were observed in this study when animals were sub-acutely exposed to cyanide with *Talinum triangulare* and *Talinum triangulare* only. Also, a marked reduction in bilirubin level was observed when group 2 animals were sub-acutely exposed to cyanide and *Vernonia amygdalina* at equivalent dose of 30 mg CN^- /kg/day for 2 weeks.

5.6 Histopathological analysis of the treatment groups

In rats dosed by gavage, highest concentrations of cyanide were found in the liver, followed by the lungs and blood (Yamamoto et al., 1982). The distribution of cyanide to the various tissues is rapid and fairly uniform, however, higher levels are generally found in liver lungs, blood, kidney and brain (ATSDR, 1997; Ballantyne, 1983; ECETOC, 2004; Gettler and Baine, 1938).

Histopathological conditions associated with sub-acute exposure of KCN can result from diminished activities of cytochrome c oxidase in the brain and rhodanese in the liver (Tulsawani *et. al*., 2005). Additionally, he also reported that KCN produced various histological changes in the brain, heart, liver and kidney.

In this study, a random selection of three animals (rats) from each experimental group for histopathological analysis was carried out. The results of the analysis of the liver, kidney, spleen and the brain revealed that, in group one (Cyanide only), there were visible lesions; multifocal and lymphocytic degeneration of the liver, congestion of blood vessels, necrosis of tubular epithelial cells and glomerular cast in the kidney,

while in the brain, a multifocal area spongiosis was presented, thereby indicating organs damage was evident with cyanide.

The rats in group two (CN + VA) after histopatological analysis had: slight fatty degeneration of the liver in a rat with no visible lesion of the kidney in all the rats and lymphoid depletion of the spleen in one of the rats selected. 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 (CN + TA), focal centriolobular hepatic necrosis and congestion of blood vessels of the kidney was observed in a rat. In experiments with rats (Ibrahim *et al.*, 1963; Lessell, 1971; Lessell and Kuwabara, 1974; Philbrick *et al.*, 1979), cats, and monkeys, selective destruction of white matter in the brain was a striking and consistent feature of poisoning from prolonged exposure to cyanide.

It is also evident in this experiment that there were no visible lesions in the liver, kidney, spleen and the brain when histopathological analysis was carried out on group four (VA only), five (TA only) and group six (control) animals suggesting that both vegetables have organs protective properties.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Vegetables play an important role in human diet they are important source of both digestible and indigestible carbohydrates. They are also important sources of minerals and certain vitamins especially vitamin A and C, they are responsible for daily well-being and protection from long term degenerative diseases (Achinewhu *et al*., 1998).

The results obtained in this study suggest that both vegetables (*Vernonia amygdalina* and *Talinum triangulare)* extracts possess detoxification properties. Both vegetables have ameliorating and protective effects on organs assessed in this study. Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans. Data in the 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 which are similar to this study showed higher production of hydrogen sulphide from cysteine than from iso- quantities of methionine or inorganic sulphate required cyanide detoxification (Bird, 1972). 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 (Bird, 1972)..

Further research work is required to carry out bioactivity guided fractionation of the plants with a view to isolate, identify and characterize the molecule(s) responsible for the observed biological activities and determine the precise mechanism of actions involved.

6.2 Recommendation

The availability of these vegetables *Vernonia amygdalina* and *Talinum triangulare* containing dietary protein capable of providing sulfane- sulphur present the opportunities of their values in the possible prevention of conditions associated with

short term sub-lethal cyanide exposure and intoxication due to their detoxification properties.

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