EFFECTS OF *GNETUM AFRICANUM* WELW. AND *OCIMUM GRATISSIMUM* LINN. AQUEOUS LEAF EXTRACTS ON THE HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN CYANIDE-TREATED *RATTUS RATTUS*

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CERTIFICATION

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DEDICATION

This work is dedicated to my Dear Father in heaven who is the Source of my wisdom, strength, inspiration and my overall being.

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ABSTRACT

Cyanide toxicity is of public health concern. Cyanide is among the most potent and deadly poisons and sources of potential human exposure to it are numerous, arising through its release into the environment from both natural and anthropogenic sources. *Gnetum Africanum* (GA) and *Ocimum Gratissimum* (OG) which contain essential amino acids required to abate cyanide toxicity effects, are widely used local plants for both nutritional and therapeutic purposes in Nigeria. There is dearth of information about ameliorating effects of these plants on cyanide toxicity. The study was aimed at determining the effects of these plants on some indices of cyanide toxicity in *Rattus rattus.*

Thirty 7-week old albino rats of same breed and similar exposure were randomly allocated to five treatment and one control groups of five rats each. Lyophilised aqueous extracts of GA and OG leaves were reconstituted in water to give a concentration of 3mg/L respectively while potassium cyanide (KCN) was prepared at 3mg/L concentration. After acclimatisation period of three weeks, the rats were randomly distributed as follow: group 1(control); group 2 (3mg/kg body weight KCN only); group 3 (3mg/kg body weight each of aqueous GA extract and KCN; group 4 (3mg/kg body weight each of aqueous OG extract and KCN); group 5 (3mg/kg aqueous GA extract only); group 6 (3mg/kg aqueous OG extract only). Treatments were administered by gavage while maintaining the rats on commercial rat pellets and water *ad libitum* for 14 days during which their body weights were noted daily. After exposure to the various treatments, biochemical analysis and haematological examination were done using the International Council for Standardisation in Haematology (ICSH) standard procedures. The results were analysed using descriptive statistics and Kruskal-Wallis at p=0.05.

The mean rat weights (g) were significantly increased in group 3 (95.5±17.3) and group 5 (98.9±11.7) in comparison with the control (88.9±17.9). Slimy nasal discharge was found in 18.6% of rats in group 2 and 10.0% of rats in group 4. No discharge was found in control, group 3, group 5 and group 6. In group 2, 17.1% of the rats had ocular lesion while other groups had no ocular lesion. Mean haemoglobin values (g/dL) were significantly lowered in group 2 (12.9±0.7), group 3 (13.0±0.5), group 4 (13.1±0.5), group 5 (12.6±0.8) and group 6 (13.2±1.5) than the control (14.4+0.7) while mean white blood cell counts (cell/□ lmm^3) were elevated with no significant difference with regards to the control. Total

protein (g/dL) were significantly lowered except in group 2 than the control. Globulin production (g/dL) was suppressed significantly in groups 2 to 4 when compared with the control. Mean creatinine values (m*g*/dL) were increased in groups 2 to 6 than the control. Mean Aspartate aminotransferase values (unit/l) were significantly reduced in group 3 (9.6±2.5) than the control (21.6±5.5).

Gnetum africanum and *Ocimum gratissimum* suppressed the haemopoietic system. *Gnetum africanum* had more deleterious effects and did not alleviate the haematologic and the biochemical effects of cyanide toxicity. The consumption of these plants with cyanoglycoside-containing food is not recommended.

Key words: Cyanide poisoning, *Ocimum gratissimum, Gnetum africanum,* Ameliorating effects.

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CHAPTER ONE

1.0 INTRODUCTION

Cyanidehas been associated with many intoxication episodes in humans and animals resulting from the ingestion of food, environmental pollution, chemical war, suicide, homicide, occupational factors and use in some drugs such as nitroprusside and laetrile (Watts, 1998). Cyanides, in the environment, arise from both man-made and naturally occurring substances. In plants, cyanide can be found mainly as cyanogenic glycosides, as found in *Manihot* sp. (cassava), *Linum* sp., *Lotus* sp., *Phaseolus lunatus, Sorghum* sp. (Conn, 1978) and the content of this substance can be high as 100-800 mg/kg of the plant material(Conn, 1978; Poulton,1983). Cyanide is among the most potent and deadly poisons, and sourcesof potential human exposure to it are numerous (WHO, 2004; Baskin and Brewer, 2006). Cyanide poisoning may result from exposure to hydrocyanic acidand cyanide salts (Benaissa *et al.*, 1995; Graham *et al.*, 1977)as well as to various cyanogenic compounds including sodiumnitroprusside (Versey, 1987) and nitriles (Bismuth *et al*., 1987;Caravati *et al.*, 1988). However, the most frequent circumstanceassociated with cyanide poisoning is smoke inhalation (Andersonand Harland, 1982; Ballantyne, 1974; Baud *et al.*, 1991; Clark*et al.*, 1981; Silverman *et al.*, 1988).

Cyanide is produced bythe thermal degradation of nitrogen-containing products foundin numerous common household materials (Alarie, 1985; Ballantyne,1974; Yamamoto, 1975; Yamamoto and Yamamoto, 1971). Existingin gaseous, solid, and liquid forms, cyanide is used in manyindustries, found in certain household substances, and producedby the combustion of common materials such as fabrics containingnylon, silk, or wool and many plastics such as melamine, polyurethane,and polyacrylonitrile(Alarie,2002; Betol *et al*., 1983). The release of cyanide and cyanogeniccompounds (such as nitriles) from combustion of such productsis the most common source of human exposure to cyanide and maybe second only in importance to carbon monoxide as a toxicantin these circumstances (Alarie,2002;Riordan *et al*.,2002; Yeoh andBraitberg,2004; Barillo *et al*.,1994; Lundquist *et al*., 1989; Baud*et al*., 1991; Walsh and Eckstein, 2004). Additional sources ofcyanide exposure include metabolites of the antihypertensivedrug nitroprusside, suicide attempts, and malicious acts suchas

murder attempts or terrorist attacks (Eckstein, 2004). Humans can also be exposedto cyanide by eating cyanogenic foods, such as the tropicalroot cassava, that contain cyanogenic glycosides that liberatecyanide when metabolized in the body.

Cyanide is a potent intracellular poison (Lawson-Smith *et al.,* 2010)which can cause deleterious effects to both man and wildlife. Cyanides are readily absorbed through inhalation, oral, and dermal routes of exposure. The toxicity of cyanide to human is dependent on the nature of the exposure. Due to the variability of dose-response effects between individuals, the toxicity of a substance is typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC_{50} or LD_{50}). The morbidity or mortality depends upon the magnitude of poisoning, which varies with the dose and form of cyanide and the route of poisoning (van Heijst*et al*., 1987; Bhattacharya,2000). The lethal dose of cyanide for an adult depends on body weight, age and nutritional status. Sometimes these limits are exceeded by persons eating a cassava meal and deaths occur due to cyanide poisoning. Smaller (non-fatal) amounts of cyanide cause acute intoxication with symptoms of dizziness, headache, stomach pains, vomiting and diarrhea.

The body has several mechanisms to effectively detoxify cyanide. The majority of cyanide reacts with thiosulfate to produce thiocyanate in reactions catalyzed by sulfur tranferase enzymes such as rhodanese. The thiocyanate is then excreted in the urine over a period of days. Although thiocyanate is approximately seven times less toxic than cyanide, increased thiocyanate concentrations in the body resulting from chronic cyanide exposure can adversely affect the thyroid (Lindsay, 2006).

Several antidotes and first aid measures which can be taken in cases of inadvertent cyanide poisoning in humans are available but they are slow and ineffective when administered after a certain point (Patterson, 2007) and their safety, efficacy and correct indication for use are frequently being debated **(**van Heijst*et al*., 1987; Bhattacharya,2000). For example some antidotes act to change normal hemoglobin to another form called methaemoglobin, as cyanide has a high affinity for this type of hemoglobin, which binds the cyanide and stops it from reaching the tissues. Unfortunately oxidized hemoglobin does not bind oxygen and so obviously it is not desirable to have too much of this form present. Extra thiosulfate can also be given so that more cyanide can be metabolized; this is often used with methaemoglobin formers.

Cobalt (II) compounds form cyanide complexes and these are used but they are highly toxic. Hydroxycobalamin (Vitamin B12a) which forms cyanocobalamin (Vitamin B12) has lower toxicity but it is not widely available.

Vegetable species are noted for their rich contents of essential amino acids, vitamins and minerals. Furthermore, they are cheap; available and widely used local plants for both nutritional and therapeutic purposes in Nigeria.Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials, and most importantly, using home vegetables in Nigeria in abating cyanide toxicity.

1.1 PROBLEM STATEMENT

Cyanide has created complex problems for modern society and these problems have evolved not only from industrial pollution but, paradoxically, from an inadvertent attempt to resolve pollution problems. Its use as a suicidal, homicidal, chemical warfare and genocidal agent is well known. Toxic problems have been associated with ingesting cyanide-containing foods, and occupational hazards have arisen as industrial use of cyanide has increased. In medicine, it has created problems because some drugs with nitrile moieties liberate cyanide. Much of the toxicological interest in cyanide has focused on its rapid action; however, its most widely distributed toxicologic problems are due to its chronic toxicity from dietary, industrial, and environmental factors. Cyanide is not wholly a toxin synthesized by civilization, as it existed in prebiotic times and was involved in biogenesis. Cyanide is produced by various organisms and plants in our environment and has a role in normal metabolism (Way, 1984).

The general population may be exposed to cyanides by inhalation of contaminated air, ingestion of contaminated drinking water, and/or consumption of a variety of foods (ATSDR, 1989). Workers in industries that use or produce cyanide compounds are at risk of exposure. Multiple casualties may present after a fire or hazardous materials incident involving cyanides. A significant risk for multiple casualties occurs when these products come into contact with mineral acids because hydrogen cyanide gas is produced. However, it does not give rise to chronic health or environmental problems when present in low concentrations. Consumers are also at risk of exposure to cyanide when using household products containing cyanide, such as pesticides. Because of prevalent use of synthetic materials in home furnishing, large amount of cyanide gas can be liberated during a house fire. People who live in areas of high motor traffic or close to chemical

other processing facilities are also at higher risk of exposure to cyanide. So too smokers and people who breathe the smoke from burning trash. Smoke inhalation and chronic poisoning affect all ages. Accidental ingestion of household substances containing poisonsoften involves young children, who place substances in theirmouths and/or ingest them as a means of exploration(Herranz and Clerigue, 2003; Michael and Sztanjnkrycer, 2004).

Cyanide poisoning, while being extremely rare, is potentially deadly. Smoke inhalation, suicidal ingestion, and industrial exposures are the most frequent sources of cyanide poisoning. Deliberate ingestion of cyanide occurs in adults acute, (sudden) exposure to cyanide in the industrial setting is the most likely cause of cyanide poisoning.
Many peasant communities in the developing countries fed on agricultural produce (plant and animal) that contain toxic substances even after processing.Among the staples in Nigerian diet for instance are cassava (*Manihot* sp.)and bitter-leaf (*Amygdaline vernonia*). Cassava contains a cyanogenic glycoside- Linamarin,while bitter-leaf contains another cyanogenic glycoside-amygdaline.In Nigeria, Cassava accounts for 41.5 per cent of the food consumed in Ogun, Oyo, and Ondo States Former Western State, as compared to 53 per cent in Bendel State (Midwest) and 45 per cent in Anambra and Imo States (East Central)(Okigbo, 1980). Cassava supplies the bulk of the energy intake in Southern Nigeria as compared to other staples; there are several cassava-based food preparations for different periods of the day and various occasions (Okigbo, 1980).

The consumption of cassava as the major source of carbohydrate has been associated with the cyanide-induced neurological disorder known as Tropical Ataxic Neuropathy(WHO, 2004).Consumption of food containing cyanogenic glycosides has been linked to several different diseases affecting mainly the nervous system, such as tropical ataxic neuropathy in Nigeria, spastic paraparesis (called mantakassa in Mozambique and konzo in the Democratic Republic of the Congo) in Cameroon, Central African Republic, Mozambique, Tanzania, and the Democratic Republic of the Congo (formerly Zaire), as well as retrobulbar neuritis and optic atrophy associated with pernicious anaemia. Cyanides have also been implicated in tobacco–alcohol amblyopia and thyroid effects such as goitre and even cretinism (Osuntokun *et al*., 1969; Ermans *et al*., 1972; Wilson, 1983; Howlett *et al*., 1990; US EPA, 1990; ATSDR, 1991; Tylleskär *et al*., 1994; Boivin, 1997; Lantum, 1998; Ernesto *et al*., 2002).

Cyanide induces fatality in seconds to minutes following inhalation or intravenous injection, in minutes following ingestion of soluble salts, or minutes (hydrogen cyanide) to several hours (cyanogens) after skin absorption. Individuals who survive cyanide poisoning are at risk for central nervous dysfunction (e.g., anoxic encephalopathy, Parkinsonlike syndrome). Excess thiocyanate due to cyanide metabolism results in a depressed uptake of iodine by the thyroid gland that may lead to symptoms of iodine deficiency, including goitre. 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. 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). 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.

Cyanide has not been reported to directly cause birth defects in people. However, birth defects occurred in rats that ate cassava root diets, and harmful effects on the reproductive system occurred in rats and mice that drank water containing sodium cyanide.

Plant protein still remains a veritable source of food nutrient for the less-privileged population in developing countries, including Nigeria where the cost of animal protein is beyond their per capita income.While exposure to cyanide has been crudely estimated to be 15–50 mg/day in endemic areas in some such cases, owing to little or no intake of the sulfur amino acids necessary for cyanide detoxification, it is therefore, of necessity to identify the health problems associated with cyanide exposure, and the use of home grown vegetables in alleviating its toxicity.

1.2 RATIONALE OF THE STUDY

Cyanide is an extremely toxic and fast-acting poison that is both widely available and easily accessible throughout the world.

There are a number of antidotes available for cyanide poisoning. However, their safety, efficacy and correct indication for use are frequently being debated **(**van Heijst*et al*., 1987; Bhattacharya,2000). According to Patterson (2007), the current cyanide antidotes work slowly and are ineffective when administered after a certain point. There is need to find a faster-acting, more effective, and better tolerated treatment for cyanide toxicity.

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acid (Okafor, 1983). Due to the toxicity of the antidotes, it is important to assess some edible vegetables, rich in essential amino acids, in abating the toxic effects of cyanide.

The rationale of this study is contingent upon the dearth of information on the ameliorating effects of these plants on cyanide toxicity.

1.3 PUBLIC HEALTH SIGNIFICANCE OF THE STUDY

Cyanide has the ability to cause significant social disruption, demands special attention and Public Health preparedness. Food and drinkable water are the main sources of cyanide exposure for individuals who are not occupationally exposed to the chemical.

The majority of the population is exposed tolow levels of cyanide in the general environment (WHO, 2004). There are, however, specific subgroups with higher potential for exposure. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogenic glycosides, such as cassava specialty foods such as apricot pits, and bitter almonds (WHO, 2004). 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 (WHO, 2004).

The public health significance of this study is based on the fact that its findings can help inform and reform decision-making process and policies on hazard control regarding cyanide poisoning. Its relevance in limiting the associated risk poisoning in the country cannot be underscored.

1.4 BROAD OBJECTIVE OF THE STUDY

The broad objective of this study is to determine the effectiveness of *Gnetum africanum* (wild spinach) and *Ocimum gratissimum* (scent leaf) in the amelioration of cyanide toxicity in populations exposed to cyanide in various forms including consumption of cyanoglycosides using rats as model.

1.5 SPECIFIC OBJECTIVES

i. To induce cyanide toxicity in rats as animal model of cyanide toxicity in human.
ii. To monitor the efficacy of*Gnetumafricanum* (wild spinach) and *Ocimum gratissimum*(scent leaf) in counteractingthe effect(s) of cyanide intoxication in the experimental animals.
iii. To extrapolate the result from two (2) above to predict the effects of each vegetable in man particularly, when ingested simultaneously with cyanide.

1.6 LIMITATION OF THE STUDY

i. The inability of obtaining the sulphur containing amino acids (methionine and cysteine) as standard references.
ii. The inability to estimate the values of the amino acids in the vegetables.
iii. The inability to monitor plasma levels of cyanide in experimental animals.
iv. The inability to monitor amount of excreted thiocyanate in the urine.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BRIEF DESCRIPTION OF CYANIDE

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

Hydrogen cyanide is a colourless or pale blue liquid or gas with a faint bitter almond-like odour (Verschueren, 1983). Hydrogen cyanide is formed during the incomplete combustion of nitrogen-containing polymers, such as certain plastics, polyurethanes, and wool. 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 present in cigarette smoke (WHO, 2004).

2.2HISTORICAL USE OF CYANIDE

Hydrogen cyanide was discovered in 1782 by Carl Scheele, who was investigating the dye Prussian blue (or Berlin blaue, as it was known in the German-speaking world). Mixing the dye with an acid and heating gave him a flammable gas that dissolved well in

water, producing an acidic solution. Logically enough, he called his discovery Berlin Blausäure (Berlin blue acid - chemical nomenclature in 1782 was not as systematic as it has become today). The Berlin was soon dropped, and it became simply Blausäure (in the English-speaking world, the name became Prussic acid). Scheele's death in 1786 is sometimes attributed to accidental poisoning by hydrogen cyanide; it may also have been due to chronic poisoning due to his habit (common among chemists of the time) of tasting everything he synthesized (Baskin and Brewer, 2006).

The actual composition - 1 atom each of hydrogen, carbon, and nitrogen - of hydrogen cyanide was not definitively determined until 1811 by Joseph-Louis Gay-Lussac. While Claude-Louis Berthollet had analyzed it in 1787 and determined that it contained only hydrogen, carbon, and nitrogen, his results had been questioned because it was manifestly an acid, and the prevailing theory of acids (Lavoisier's oxygen theory of acidity) at the time stated that oxygen had to be present for a material to act as an acid. Gay-Lussac's results for hydrogen cyanide, along with those of Davy for hydrochloric acid, were essential in disproving this theory.

Its poisonous properties led to its early consideration as a chemical warfare agent, but during the First World War, hydrogen cyanide was employed only occasionally, primarily by the French who dubbed it Forestite. Because of its high vapor pressure and low vapor density it tended to dissipate rapidly, and its low flash point meant that it would often (about half the time) ignite when released from artillery shells, limiting its military effectiveness. The French attempted to produce hydrogen cyanide-containing mixtures that would be more persistent, and so more useful. The best known of these is probably Vincennite, which was a mixture of 50% hydrogen cyanide with the smoke producers arsenic trichloride (30%) and stannic chloride (15%) along with chloroform as a stabilizer. Despite their best efforts, however, they were never able to produce a hydrogen cyanide munition that answered the needs of the period, and in a war in which the chemical industries of the world strained to produce enough deadly chemicals, usage of hydrogen cyanide was a relatively paltry 4000 tons.

The French believe in the utility of hydrogen cyanide as a war gas stemmed in large part from experiments done on inhalation exposure in dogs (IPCS, 1993). There was considerable disagreement in the chemical warfare community over how (or whether) the data from the dog experiments applied to humans - British experiments with goats had led

them to the opposing conclusions about its utility. This disagreement led to one of the more dramatic (and foolhardy) moments in the history of research on chemical warfare agents. A British researcher, Joseph Barcroft, emphatically disagreed with the French estimates of lethality. In order to prove his point, he sneaked into a testing chamber at Porton Down along with a dog but without a gas mask (although he did bring along a witness who stayed outside the chamber). Hydrogen cyanide was introduced into the chamber to give a concentration of about 500 mg m^{-3}. Barcroft gave this description of what happened:

In order that the experiment might be as fair as possible and that my respiration should be relatively as active as that of the dog, I remained standing, and took a few steps from time to time while I was in the chamber. In about thirty seconds the dog began to get unsteady, and in fifty-five seconds it dropped on the floor and commenced the characteristic distressing respiration which heralds death from cyanide poisoning. One minute thirty-five seconds after the commencement the animal's body was carried out, respiration having ceased and the dog being apparently dead. I then left the chamber.

This experiment, combined with the existing perception that hydrogen cyanide dispersed too rapidly to produce an effective concentration, meant that hydrogen cyanide was largely ignored as a military agent by most countries after the First World War. It is also frequently cited as an example of the hazards of extrapolating from animal models to humans.

Nevertheless, the Japanese investigated hydrogen cyanide. Tests were conducted on human subjects, including in at least one case a small child, in China at the facilities of the notorious unit 731 in conjunction with the Chemical Warfare Unit 516 using a telephone box-sized chamber. There is also evidence that Japanese forces may have used hydrogen cyanide or a related agent in chemical attacks in China on as many as 36 occasions between 1938 and 1941. It would, however, remain a very minor agent in the Japanese inventory, with about 250 tons being produced for the Imperial Japanese Army between 1930 and 1945.

Only the Soviet Union would invest significantly in weapons based on hydrogen cyanide. They had figured out that if you used a big enough charge in your bomb you got evaporative cooling that would allow production of an effective concentration at ground

level. They had also developed spray tanks that allowed dissemination of enough agents to produce an effective concentration at ground level (with low altitude spraying). The Soviets also saw an advantage to the use of hydrogen cyanide in that most gas mask filters of the day worked relatively poorly against it. While the Soviets, like most countries, intended to use mustard gas as their main agent, they produced a significant quantity of hydrogen cyanide - figures are difficult to come by, but wartime (1940-1945) production totaled at least 11,104 tons.

During the Second World War, the Germans captured some Soviet spray tanks and tested them at their facility at Munsterlager, and were considerably disturbed by what they found - both unprotected animals and animals inside tanks (the panzer kind) died after about a few minutes. The German gas mask was also poor protection against this particular agent, with the FE-41 filter failing in about an hour, and the FE-42 in about an hour and forty-five minutes (in contrast, the MT-4 mask the Soviets were issuing would last about 8 hours). This knowledge probably served as a disincentive for German use of chemical weapons in the East.

The most significant use of hydrogen cyanide during World War II was in the concentration camps. The principle agent used to murder those that the Nazi regime deemed undesirable was Zyklon B, which was hydrogen cyanide (40% by weight) absorbed on a calcium sulfate carrier(Baskin and Brewer, 2006).

After the Second World War, the importance of hydrogen cyanide as a chemical warfare agent diminished rapidly, primarily as a result of the rise of nerve agents. Even the Soviets, who had made themselves the masters of its use, relegated it to the third tier of their chemical arsenal, classifying it as a reserve agent.

But, although reduced in importance, allegations of its use still crop up from time to time. For instance, in February of 1985, the government of Thailand complained to the UN that rockets containing phosgene gas and hydrogen cyanide launched by Vietnamese forces fighting Cambodian rebels had landed on Thai territory. More famously, hydrogen cyanide is supposed to have been one of the agents in the chemical cocktail used against Halabja in the chemical assault carried out by Iraqi forces on that city on March 16-19, 1988. (This allegation remains controversial, as the cyanide residues on which it is based could also have arisen from impurities in the Tabun that was definitely used and the mix of agents employed makes identifying it from symptoms impossible. It has also been

suggested that hydrogen cyanide may have come from an Iranian, rather than an Iraqi, chemical attack.

2.3 PROPERTIES OF CYANIDE

2.3.1. IDENTITY ANDPHYSICAL PROPERTIES

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

- The hexacyanides $[M(CN)_6]^{3-}$ (M = Ti, V, Cr, Mn, Fe, Co), which are octahedral in geometry;
- The tetracyanides, $[M(CN)_4]^{2-}$ (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:

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

Metal catalysts are required for such reactions.

Hydrogen cyanide is a colourless or pale blue liquid with characteristic odour of bitter almond (Verschueren, 1983). Common synonyms are hydrocyanic acid and prussic acid. Hydrogen cyanide is a very weak acid, with a p*K*a value of 9.22 at 25 °C. It is soluble in water and alcohol. Hydrogen cyanide is commercially available as a gas or as a technical-grade liquid in concentrations of 5, 10, and 96–99.5%. It has a molecular weight of 27.03 and a boiling point of 25.6^0C (Amooreand Hautala, 1983). It is miscible with water and alcohol and slightly soluble in ether (Budavari *et al*., 1989).Phosphoric acid is added to liquid hydrogen cyanide as a stabilizer to prevent decomposition and explosion (ATSDR,

1997). The conversion factor for hydrogen cyanide in air (at 20 °C and 101.3 kPa) is as follows:

1 ppm = 1.12 mg/m3

1 mg/m3 = 0.890 ppm

Most people can smell hydrogen cyanide. Due to an apparent genetictrait, 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:

$NaCN + H_2O \rightarrow HCN + NaOH$

Sodium cyanide (NaCN) is a white hygroscopic crystalline powder with a faint bitter almond-like odour. Common synonyms are cyanide of sodium and hydrocyanic acid, sodium. Commercially available sodium cyanide generally achieves a purity of 95–98%. The aqueous solution of sodium cyanide is strongly alkaline and rapidly decomposes. Sodium cyanide produces hydrogen cyanide on contact with acids or acid salts.

Potassium cyanide (KCN) is a white deliquescent solid with an odour of hydrogen cyanide. Common synonyms are hydrocyanic acid, potassium salt and cyanide of potassium. Potassium cyanide is commercially available at95% purity. An aqueous solution of potassium cyanide in water is strongly alkaline. Potassium cyanide also produces hydrogen cyanide on contact with acids or acid salts.

Calcium cyanide ($Ca(CN)_2$), also commonly called cyanide of calcium, calcid, or calsyan, is a white crystalline solid. Its aqueous solution gradually liberates hydrogen cyanide. Cyanides such as sodium cyanide, potassium cyanide, and calcium cyanide form strong complexes with many metals.

Cyanogen is a colourless toxic gas with an almondlike odour. Common synonyms are carbon nitrile, dicyanogen, ethane dinitrile, and oxalic acid dinitrile. Cyanogen is slowly hydrolysed in aqueous solution, yielding oxalic acid and ammonia. The conversion factors for cyanogen in air at 20 °C and 101.3 kPa are asfollows:

1 ppm = 2.16 mg/m3

1 mg/m3 = 0.462 ppm

2.3.2 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(ICMI, 2006). 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(ICMI, 2006). 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(ICMI, 2006). However, iron cyanides are subject to photochemical decomposition and will release cyanide if exposed to ultraviolet light(ICMI, 2006).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 ($CuFeS_2$), chalcocite (Cu_2S) 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).

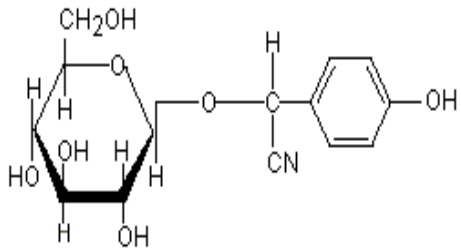
2.4 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE OF CYANIDE

2.4.1 Natural occurrence

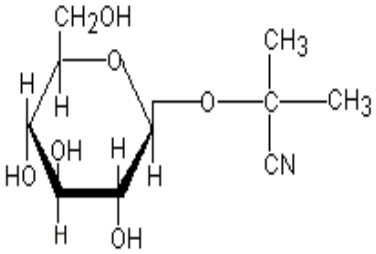
Hydrogen cyanide is ubiquitous in nature. It is found in the stratosphere and non-urban troposphere (US EPA, 1990). It is released into the atmosphere from biomass burning, volcanoes, and natural biogenic processes from higher plants, bacteria, algae, and fungi (Fiksel *et al*., 1981; Cicerone & Zellner, 1983; Way, 1984; ATSDR, 1997; Li *et al*., 2000). An estimate of the amount of cyanide released to the environment from natural biogenic processes is not available (ATSDR, 1997). Cyanide occurs naturally as cyanogenic glycosides in at least 2000 plants (JECFA, 1993). Known cyanogenic glycosides in plants include amygdalin, linamarin, dhurrin, prunasin, lotaustralin and taxiphyllin. Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-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).

After ingestion, linamarin can be hydrolysed by either cassava linamarase or an endogenous β-glucosidase to yield D-glucose and ACH (Frakes*et al*., 1986a).

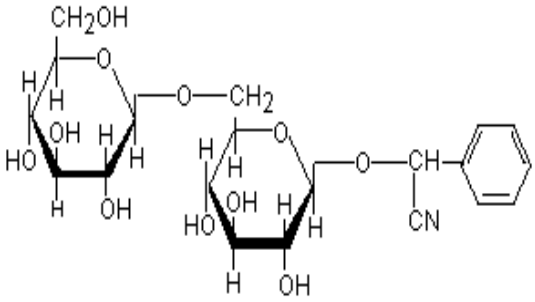
Dhurrin (CAS No. 499-20-7)



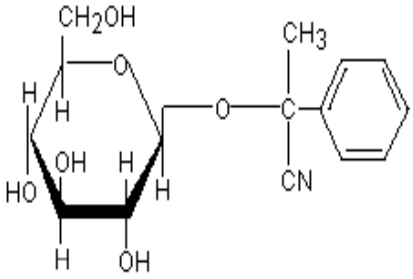
Linamarin (CAS No. 554-35-8)



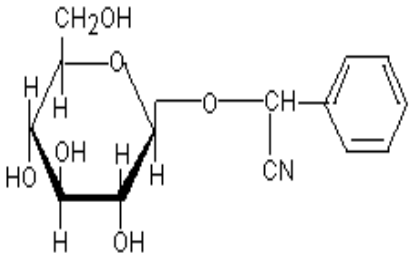
Amygdalin (CAS No. 29883-15-6)



Lotaustralin (CAS No. 534-67-8)



Prunasin (CAS No. 99-18-3)



Taxiphyllin (CAS No. 21401-21-8)

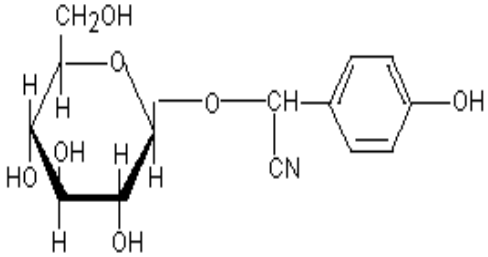


Figure 2.1: Cyanogenic glycosides in major edible plants (JECFA, 1993)

2.4.2 Anthropogenic sources

2.4.2.1 Production

Hydrogen cyanide is principally produced by two synthetic catalytic processes involving the reaction of ammonia and natural gas (or methane) with or without air. It is also obtained as a by-product in the production of acrylonitrile by the ammoxidation of propylene, which accounts for approximately 30% of the worldwide production of hydrogen cyanide. Sodium and potassium cyanides are principally prepared by the direct reaction of hydrogen cyanide with the respective alkali in closed systems (European Chemicals Bureau, 2000a, b). Sodium cyanide is also prepared to a lesser extent by melting sodium chloride with calcium cyanamide or by heating sodium amide salt with carbon. Calcium cyanide is produced by the reaction of coke, coal, and limestone. Cyanogen chloride is 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 (WHO, 1996; IPCS, 2000a). Acetone cyanohydrin(ACH) was first produced in the 1930s as an intermediate in the production of methyl methacrylate from hydrogen cyanide. It is currently produced from the liquid-phase reaction of hydrogen cyanide and acetone in the presence of an alkali catalyst at atmospheric pressure (ECETOC, 2004).

Hydrogen cyanide capacity is generally treated as the sum of purposeful direct synthesis and that derived as a by-product of acrylonitrile production. Annual US hydrogen cyanide capacity by 11 companies in 1991 was 666 000 tonnes. US production of hydrogen cyanide from 1983 to 1989 rose from 300 000 to 445 000 tonnes (Pesce, 1993). Output of hydrogen cyanide in the USA was 545 000 tonnes in 1992 (Cohrssen, 2001). Worldwide annual production and capacity of hydrogen cyanide in 1992 were estimated to be 950 000 and 1 320 000 tonnes, respectively (Pesce, 1993; Cohrssen, 2001). It has been estimated that the present total annual production of hydrogen cyanide worldwide is 1.4 million tonnes (Mudder & Botz, 2000).

2.4.2.2 Use

In 1983, the major end uses of hydrogen cyanide in the USA were in the production of adiponitrile (200 000 tonnes), ACH (128 000 tonnes), cyanuric chloride (28 500 tonnes), sodium cyanide (69 000 tonnes), chelating agents (15 800 tonnes), and nitrilotriacetic acid (10 100 tonnes) and for miscellaneous uses (20 000 tonnes) (US EPA, 1990). Hydrogen cyanide is also used in the production of methyl methacrylate, methionine and

its hydroxylated analogues, and potassium cyanide (ATSDR, 1997; ECETOC, 2004). Sodium cyanide is extensively employed in a large number of industrial processes, including electroplating and case-hardening of metals; the extraction (cyanidation) of gold and silver from ores; base metal flotation; coal gasification; and the fumigation of ships, railroad cars, buildings, grain silos, flour mills, seeds in vacuum chambers, and soil. Large quantities of sodium cyanide are used to introduce cyano groups into organic compounds, in particular through a reaction with organic halogen compounds to yield nitriles. The nitriles can then be converted to a variety of carboxylic acids, amides, esters, and amines. Potassium cyanide is usedfor electrolytic refining of platinum, for metal colouring, and as an electrolyte for the separation of gold, silver, and copper from platinum (Eisler et al., 1999; Patnaik, 1999; ACGIH, 2001; ECETOC, 2004). Cyanide salts are used as chelating agents, and the complex cyanides of copper, zinc, and cadmium are used in electroplating processes, principally the plating of iron, steel, and zinc (ECETOC, 2004). Calcium cyanide is used chiefly as a fumigant, because it readily releases hydrogen cyanide when exposed to air; as a fertilizer, defoliant, herbicide, and rodenticide; as a stabilizer for cement; and in stainless steel manufacture (ACGIH, 2001).

Cyanogen is used as a fumigant, as a fuel gas forwelding and cutting heat-resistant metals, and as a rocket and missile propellant (ATSDR, 1997). Cyanogen chloride is used as a fumigant gas and as a reagent in chemical synthesis. Cuprous cyanide is used in plating baths for silver, brass, and copper–tin alloy plating (ATSDR, 1997), as an antifouling agent in marine paint, and as an insecticide and fungicide (Windholz, 1983). Potassium silver cyanide is used in silver plating and as a bactericide. Potassium ferricyanide is used chiefly for blueprints, in photography, for staining wood, in calico printing, and in electroplating. Sodium ferrocyanide is used in ore flotation, as an anti-caking agent in rock salt, and in photography for bleaching, toning, and fixing. Sodium nitroprusside has been used as an antihypertensive agent and in congestive heart failure and is used for deliberate induction of hypotension during certain neurosurgical procedures (WHO, 2004). ACH is used in preparative transcyanohydrination reactions (WHO, 2004).

2.4.2.3 Release to the Environment

More than 30 large-scale accidental releases of cyanide to water systems have been reported since 1975; these include transportation accidents, pipe failures, and tailings dam-related releases (Korte *et al*., 2000; Mudder & Botz, 2000).

Non-point sources of cyanide released to water can result from runoff from cyanide-containing anti-caking salts (i.e., sodium ferrocyanide) used on roads, migration from landfills, and agricultural and atmospheric fallout and washout (ATSDR, 1997). The extraction of gold from low-grade ores by cyanidation processes was estimated to result in a worldwide emission of 20 000 tonnes of hydrogen cyanide into the atmosphere (Korte & Coulston, 1998). Another estimate suggested that currently 45 300 tonnes of cyanide are used in the USA in the cyanidation process. The wastes from these processes result in large cyanide-containing ponds near the mining operations (Clark & Hothem, 1991; Henny *et al*., 1994; Ma & Pritsos, 1997; Eisler *et al*., 1999). The major point sources of cyanide release to water are discharges from gold mining plants, publicly owned wastewater treatment plants, iron and steel production, and the organic chemical industries.

An estimated 3 billion litres (i.e., 3 × 109 litres) of wastes containing cyanides were generated in the USA in 1983, principally from spent cyanide plating bath solutions from electroplating operations (except for precious metals) and from spent stripping and cleaning bath solutions from electroplating operations (Grosse, 1986). During cassava starch production, large amounts of cyanoglycosides are released and hydrolysed by plantborneenzymes, leading to cyanide concentrations in wastewater as high as 200 mg/litre (Siller & Winter, 1998). The major sources of cyanide released to air, in addition to exhaust from vehicle emissions, are diverse, including chemical manufacturing (hydrogen cyanide, methyl methacrylate, acrylonitrile); processing industries, such as metallurgical industries and metal plating (i.e., electroplating metals and finishing [metal polishes]); extraction of gold and silver from low-grade ores; volatilization from cyanide wastes disposed of in landfills and waste ponds; the production of coke or other coal carbonization procedures; emissions from municipal solid waste incinerators; and direct release ofcyanides to the atmosphere resulting from fumigation operations, combustion of polyurethanes, acrylonitrile, and polyamide plastics, and combustion of wool, silk, and fibres (Carotti & Kaiser, 1972; Fiksel *et al*., 1981; ATSDR, 1997; Eisler *et al*., 1999).

An estimated total of 1 million tonnes of hydrogen cyanide, amounting to 73.1% of the total environmental releases in the USA, was discharged to the air from manufacturing and processing facilities (ATSDR, 1997). The estimated amounts of hydrogen cyanide released to air in 1976 from the most common nonindustrial sources were as follows: agricultural pest control, 62 tonnes; incineration, 8.2–82 tonnes; and tobacco smoke, 5.9–340 tonnes (Fiksel *et al*., 1981; ATSDR, 1997). In 2001, from various locations in the USA, about 1300 tonnes of hydrogen cyanide were released on- and off-site; 540 tonnes were emitted to the atmosphere,0.1 tonne was released to surface waters, 770 tonneswere injected into Class I wells, 1 and 0.42 tonne was released to land (US EPA, 2003c). In 2001, from various locations in the USA, approximately 3400 tonnes of cyanides (not otherwise specified) were released on- and off-site; 220 tonnes were emitted to the atmosphere, 47 tonnes were released to surface waters, 1800 tonnes were injected into Class I wells, and 1300 tonnes were released to land (US EPA, 2003c). Hydrogen cyanide has been found following thecombustion of a number of synthetic polymers.

The maximum yield of hydrogen cyanide per gram of polyurethane foam ranged from 0.37 to 0.93 mg under nonflaming conditions and from 0.5 to 1.02 mg under flaming combustion (Sklarew & Hayes, 1984). Hydrogen cyanide concentrations in the off-gas from the shale oil retorting process ranged from 7 to 44 mg/m3 (Sklarew & Hayes, 1984). One cigarette without a filter liberates 500µg hydrogen cyanide, while filter cigarettes liberate only 100µg 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). 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 byproduct of the chloramination of water (e.g., via the reaction of humic substances with chlorine and chloramines used for water disinfection) (Ohya & Kanno

1987; WHO, 1996; IPCS, 2000a). 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, 2002). Cyanogen is generated in the combustion of nitrogen–carbon compounds and appears in automobile exhaust gases and gases from blast furnaces (CHEMINFO, 1998).

Cyanide is present in the air mostly as a gas, and cyanides have the potential to be transported over long distances from their respective emission sources.

2.5 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE TO CYANIDE

2.5.1 Environmental levels

2.5.1.1 Air

Cyanide is found in ambient air as hydrogen cyanideand to a smaller extent in particulate matter. Theconcentration of hydrogen cyanide measured since 1981in the northern hemisphere's non-urban troposphereranged from 180 to190 ng/m^3 (Cicerone & Zellner,1983; Jaramillo *et al*.,1989).Ambient air monitoring data for cyanides in Bulgariain areas near petrochemical plants showed concentrationsranging from 0.2 to 0.8 µg/m^3 (annual averagevalue) (Kaloyanova et al., 1985).Cyanide has been detected at levels of 20–46 mg/m^3in the air near large-scale cassava processing facilities inNigeria (Okafor & Maduagwu, 2000).

2.5.1.2 Water

Cyanides, reported as cyanide, hydrogen cyanide, sodium cyanide, potassium cyanide, calcium cyanide, or copper (I) cyanide, have been detected in surface water samples at 70 of the 154 hazardous waste sites where they were studied in the USA; they have also been detected in groundwater samples at 191 of the 419 waste sites studied and in leachate samples of 16 of the 52 sites studied. The median concentrations in the positive samples were 160µg/litre for groundwater, 70µg/litre for surface water, and 479µg/litre 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 3.5µg/litre. Data from the late 1970s to early 1980s indicated that the levels are higher only in limited areas and may exceed 200µg/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 10µg/litre (US EPA,1993a). Cyanogen chloride is one of the 18 compounds that occur most frequently (8 of 10 city surveys) in potable water within the framework of the US National Organic Reconnaissance Survey (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).

2.5.1.3 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).

2.5.1.4 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). 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).

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
Lima beans from Java (colored)	3000
Lima beans from Puerto Rico (black)	2900
Lima beans from Burma (white)	2000

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

Table 2.1 shows the ranges of cyanide concentrations in some plants and plant products (i.e., Cereals and their products, soy protein products, and apricot pits). Hydrogen cyanide can be produced by hydrolytic reaction catalysed by one or more enzymes from the plants containing cyanogenic glycosides. In kernels, for example, this reaction is catalysed by the enzyme emulsin (Lasch & El Shawa, 1981) when the seeds are crushed and moistened. Amygdalin (which is also present in cassava, bitter almonds, and peach stones) is converted to glucose, benzaldehyde, and hydrogen cyanide (Figure 2.2) (IPCS, 1992). Hydrogen cyanide release can occur during maceration, which activates intracellular β-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, andthe hydrolysis is complete in 10 min. Hydrolysis is also possible in an acid solution but 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 & El Shawa, 1981).

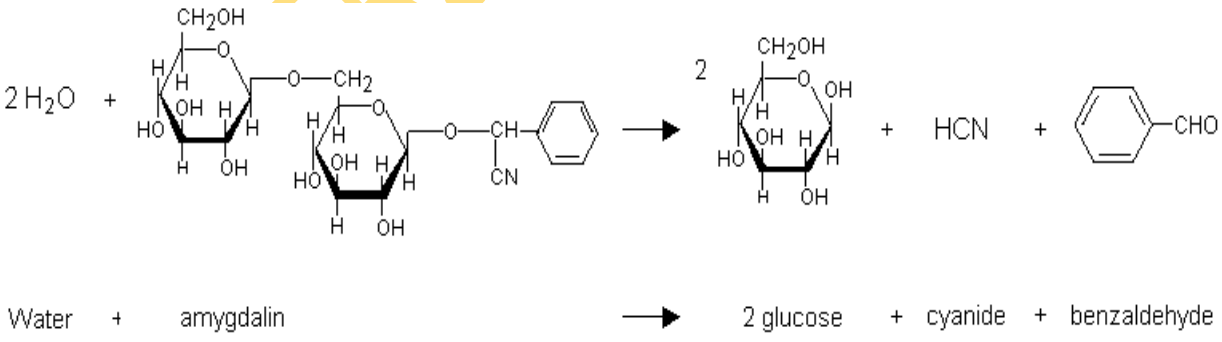


Figure 2.2: Hydrolysis of amygdalin

2.5.1.5 Other

Laetrile (another name for amygdalin derived from apricot kernels), which was formerly used as an anticancer agent, releases cyanide upon metabolism. Bitter almonds and apricot pits containing cyanogenic glycosides are still sold in health food stores and over the Internet (Suchard *et al*., 1998). Other drugs, such as sodium nitroprusside, which is used as an antihypertensive and in congestive heart failure (Guiha *et al*., 1974; Tinker, 1976; Aitken *et al*., 1977; Schultz, 1984; Rindone & Sloane 1992), also liberate hydrogen cyanide in the body. In sodium nitroprusside, the CN– moiety represents 44% by weight of the molecule. Some aliphatic nitriles that are widely used in the chemicalindustry — i.e., acetonitrile (IPCS, 1993), acrylonitrile (IARC, 1999), succinonitrile, and adiponitrile — also release cyanide upon metabolism (Willhite & Smith, 1981).

2.6 HUMAN EXPOSURE

2.6.1 General population

The general population may be exposed to cyanide from ambient air, drinking-water, and food. Based on an atmospheric hydrogen cyanide concentration of 190ng/m^3 and an average daily inhalation of 20m^3 air, the inhalation exposure of the general US non-urban, non-smoking population to hydrogen cyanide is estimated to be 3.8µg/day (ATSDR, 1997). Based on a daily drinking-water consumption of 2 litres for an adult, the daily intake of cyanogens chloride is estimated to be 0.9–1.6 µg (equivalent to 0.4– 0.7 µg of cyanide) (ATSDR, 1997) for cyanogens 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. 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. 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, 1984b). 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). Urinary excretion of thiocyanate has been applied in the biological monitoring of exposure to cyanogenic glycosides, especially among cassava-consuming populations. The average urinary thiocyanate concentration among children in the Bandundu region of the Democratic Republic of the Congo (formerly Zaire) was 757µmol/litre in the south and 50µmol/litre in the north (both populations consumed cassava as their staple diet, but the cassava was well processed in the north and inadequately processed in the south). These concentrations can be compared with an average of 31µmol/litre in a non-smoking Swedish reference population (Banea-Mayambu *et al*., 2000). In the same Bandundu region, it was shown that there was a marked seasonal variation in urinary thiocyanate concentrations in the villages with a high "konzo" (spastic paraparesis) incidence (563–627µmol/litre in the dry season and 344–381 in the wet season), while the average in non-konzo areas was 241µmol/litre (Banea-Mayambu et al., 1997). In Mozambique, the average urinary thiocyanate levels among healthy children from areas with epidemic spastic paraparesis varied between 33 and 1175 µmol/litre, whereas levels in areas with no paraparesis were between 18 and 400 µmol/litre (Casadei *et al*., 1990). In Nampula province in Mozambique, where spastic paraparesis epidemics had been observed in 1981–1982 and during the civil war in 1992–1993, average urinary thiocyanate concentrations among schoolchildren in five areas were between 225 and 384 µmol/litre in October 1999 (Ernesto et al., 2002). In Malawi, in an area where cassava was typically soaked for 3–6 days for processing to flour, urinary thiocyanate concentrations were between 2 and 410µmol/litre, with a median of 32µmol/litre (Chiwona-Karltun et al., 2000).

2.6.2 Occupational exposure

The principal routes of occupational exposure to cyanides are via inhalation and, to a lesser degree, skin absorption. Skin absorption may be significant under some circumstances — for example, when airborne concentrations are very high, such as in fumigation operations. It may occur as well when personal protection is inadequate and operators are splashed. Workers involved in electroplating, metallurgy, pesticide application, firefighting, gas works operations, tanning, blacksmithing, metal cleaning, photoengraving, photography, and the manufacture of steel, cyanides, adiponitrile and other nitriles, methyl methacrylate, cyanuric acid, dyes, pharmaceuticals, or chelating

agents have the potential to be occupationally exposed to higher concentrations of cyanide (Prohorenkov & Kolpakov, 1978; Philips, 1989; IPCS, 1992; Banerjee *et al*., 1997). A number of illustrative levels of cyanide in the breathing zone of workers in working environments monitored at different production facilities in the USA during the period 1976–1982 have been reported. The concentration of cyanide in air at a plating facility of a national airline was 0.001–0.004mg/m3 (NIOSH, 1982). Concentrations of hydrogen cyanide in air at a plating facility of an electrical and electronic company in Virginia, USA, ranged from 0.07 mg/m3 in a salt pot room to 4.3 mg/m3 in a stripping tank (NIOSH, 1976).The concentration of cyanide in air at a plating facility in Ohio, USA, was 1.7mg/m^3 (NIOSH, 1978).

2.7COMPARATIVE KINETICS AND METABOLISM OF CYANIDE IN LABORATORYANIMALS AND HUMANS

2.7.1 Absorption

Hydrogen cyanide and other cyanide salts, is readily absorbed following inhalation, oral, and dermal exposure. Following exposure to cyanide in the atmosphere, toxic amounts of cyanide are absorbed with great rapidity through the bronchial mucosa and alveoli (ATSDR, 1997). Humans retained 58% of the hydrogen cyanide in the lungs after inhaling the gas through normal breathing (Landahl & Herrmann, 1950; ATSDR, 1997). Alkali metal cyanides are rapidly absorbed from the gastrointestinal tract. The presence of food in the gut, the pH of the gut, and the lipid solubility of the cyanide compound affect absorption. Gastrointestinal absorption of inorganic cyanide salts is slower than pulmonary absorption, and the onset of symptoms is delayed and the severity of symptoms diminished compared with inhalation (WHO, 2004). When simple cyanide salts such as potassium and sodium cyanide are ingested, free cyanide ion can rapidly bind hydrogen ion to form hydrogen cyanide in the highly acidic medium of the stomach. Essentially all cyanide ingested as cyanide salts will form hydrogen cyanide and will be quickly absorbed. However, after oral intake, only part of the dose reaches the blood due to first-pass metabolism by the liver (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).

2.7.2 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 & Baine, 1938; Ballantyne, 1983a; ATSDR, 1997; ECETOC, 2004). In contrast, high proportions of ingested sodium and potassium cyanide will pass through the liver and are detoxified by the first-pass effect. The major portion of cyanide in blood is sequestered in the erythrocytes, and a relatively small proportion is transported via the plasma to target organs. Cyanide is concentrated in red blood cells at a red blood cell to plasma ratio of 199:1; levels in plasma reflect tissue levels better than levels in whole blood or erythrocytes. Small but significant levels of cyanide are found in normal blood plasma (<140 µg/litre) and other tissues (<0.5 mg cyanide/kg) of humans without known occupational cyanide exposure (Feldstein & 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 106µg/litre, with a mean of 48µg/litre (Feldstein &

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

In rats dosed by gavage, highest concentrations of cyanide were found in the liver, followed by the lungs and blood (Yamamoto *et al*., 1982). 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 goitre and cretinism (Nahrstedt, 1993). A number of illustrative levels of cyanide in organs and blood after oral intake in humans (Ansell & Lewis, 1970; ATSDR, 1997) and rabbits (Ballantyne, 1983a) have been reported. For a given exposure route, whole blood and serum cyanide levels are quite similar for different species (Ballantyne, 1983a).

2.7.3 Kinetics of Cyanide and Health Effects in Human

Cyanide is produced in the human body and exhaled in extremely low concentrations with each breath. It is acutely toxic to humans. Liquid or gaseous Hydrogen cyanide and alkali salts of cyanide can enter the body through inhalation, ingestion or absorption through the eyes and skin. The rate of skin absorption is enhanced when the skin is cut, abraded 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, 1991). Typical signs of acute cyanide poisoning include tachypnoea, headache, vertigo, lack of motor coordination, weak pulse, cardiac arrhythmias, vomiting, stupor, convulsions, and coma (Ballantyne, 1983b; Way, 1984; Johnson & Mellors, 1988). Pathological findings may include tracheal congestion with haemorrhage, cerebral and pulmonary oedema, gastric erosions, and petechiae of the brain meninges and pericardium (Way, 1984). Sequelae of severe acute cyanide exposure may also include Parkinson-like syndromes and cardiovascular signs of delayed post-

hypoxic myocardial lesions, as well as neuropsychiatric manifestations similar to those seen with post-hypoxic post-carbon monoxide encephalopathy (ATSDR, 1991). Dermal absorption of hydrogen cyanide is much slower than pulmonary absorption, and the amount and speed of absorption through human skin are dependent on the amount of skin moisture and duration of skin contact. The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure. Due to the variability of dose-response effects between individuals, the toxicity of a substance is typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC_{50} or LD_{50}). The LC_{50} for gaseous hydrogen cyanide is 100-300 parts per million. Inhalation of cyanide in this range results in death within 10-60 minutes, with death coming more quickly as the concentration increases. Inhalation of 2000ppm hydrogencyanide causes death within one minute. The LD_{50} for ingestion is 50-200 milligrams or 1-3 milligrams per kilogram of body weight, calculated as hydrogen cyanide. For contact with unabraded skin, the LD_{50} is 100 milligrams (as hydrogen cyanide) per kilogram of body weight. An average LD_{50} value for dermal exposure of 100 mg/kg body weight was estimated for humans (Rieders, 1971). Although the time, dose and manner of exposure may differ, the biochemical action of the cyanide is the same upon entering the body. Once in the blood stream, cyanide forms a stable complex with a form of cytochrome C oxidase, an enzyme that promotes the transfer of electrons in the mitochondria of cells during the synthesis of ATP. Without proper cytochrome oxidase function, cells cannot utilize the oxygen present in the blood stream, resulting in cytotoxic hypoxia or cellular asphyxiation. The lack of available oxygen causes a shift from aerobic to anaerobic metabolism, leading to the accumulation of lactate in the blood. The combined effect of the hypoxia and lactate acidosis is depressed in the central nervous system that can result in respiratory arrest and death. Following oral administration to rats, LD_{50}s of hydrogen cyanide, sodium cyanide, and potassium cyanide are very similar: 0.156, 0.117, and 0.115 mmol/kg body weight, respectively, i.e., 3-4 mg cyanide/kg bodyweight (Ballantyne, 1983a1). At higher lethal concentrations, cyanide-poisoning also affects other organs and system in the body including the heart. There are no qualitative differences in acute poisoning between cyanide compounds, since the cyanide ion is the common agent that primarily inhibits tissue cytochrome oxidase activity in rats, mice, and rabbits, with resulting anoxia (Way, 1984; US EPA, 1988).

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; EPA, 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- aminothiazoline- 4-carboxilic acid with other minor metabolites (ATSDR, 1989).

2.7.4 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 2.3) (Williams, 1959; Ansell & Lewis, 1970). This route detoxifies about 80% of cyanide (Diasolua-Ngudi, 2005). The rate-limiting step is the amount of thiosulfatewhich is produced by ß-mercaptopyruvate resulting from transamination of cysteine(Diasolua-Ngudi, 2005). 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 per milligram of mitochondrial protein basis) than in the liver (Dahl, 1989). Dogs have a lower overall activity of rhodanese than monkeys, rats, and rabbits (ATSDR, 1997).

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.*, 1982; 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 & Rickards, 1952) and the non-enzymatic combination of cyanide with cysteine, forming 2-aminothiazoline-4-carboxylic acid, which appears to be excreted without further change (Rieders, 1971) (Figure 2.3).

In studies with rats orally administered potassium cyanide and maintained for up to 4 weeks on either a balanced diet or a diet lacking the sulfur amino acids L-cystine and L-methionine, a strongly positive linear relationship was found between blood cyanide and plasma cyanate (OCN^-) concentration (Tor-Agbidye *et al*., 1999). It was suggested that in Africa, where there are protein-deficient populations whose levels of sulfur-containing amino acids are low(WHO, 2004),cyanide (from prolonged use of cassava) may conceivably be converted to cyanate(Tor-Agbidye *et al*., 1999), 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 & 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 *et al*., 1982), which is considerably slower than in small rodents (Schubert and Brill, 1968) or dogs (Lawrence, 1947).

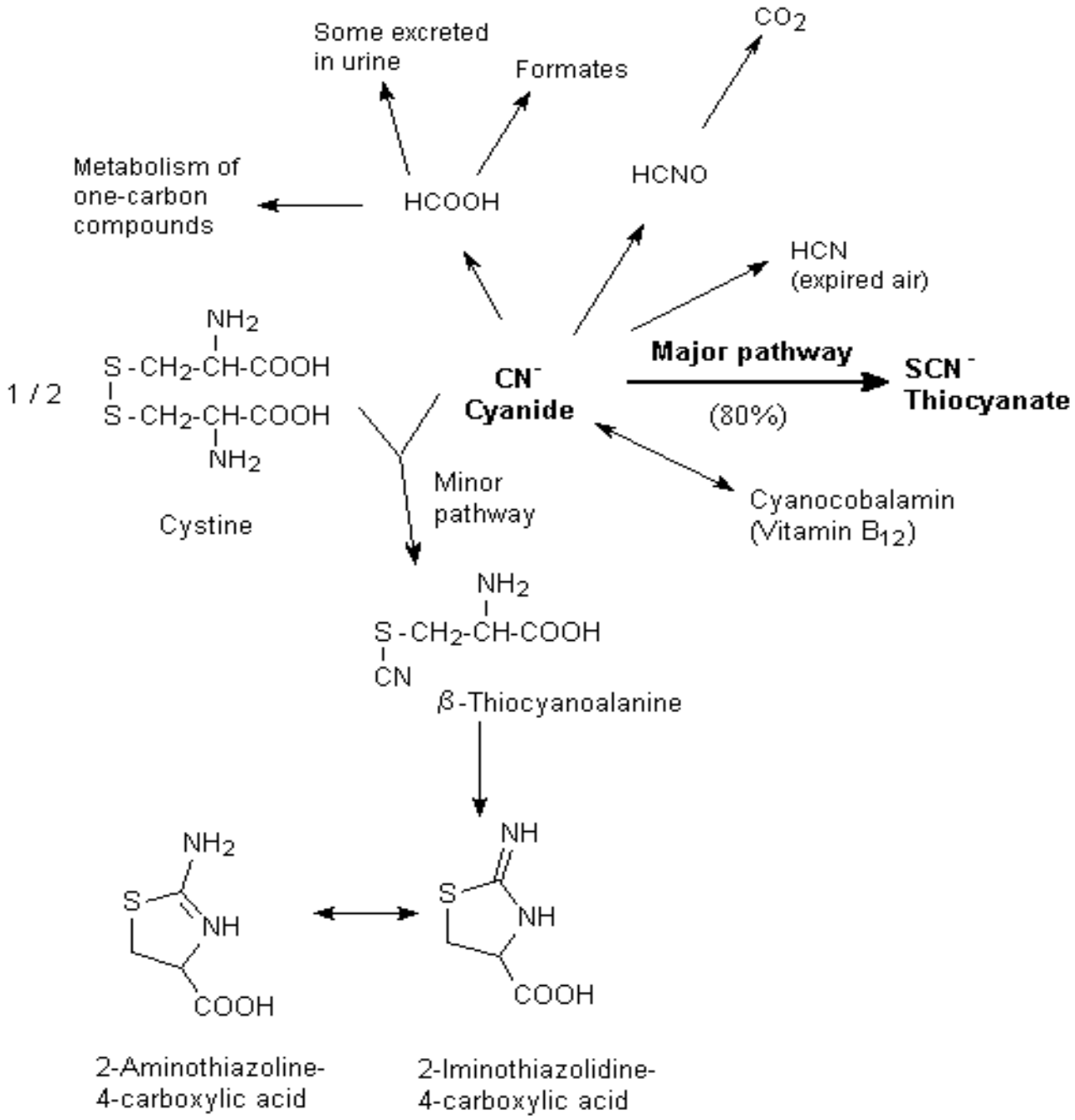


Figure 2.3: Basic processes involved in the metabolism of cyanide (ATSDR, 1997)

2.8 TOXICITY

The toxic effects of cyanide in humans and animals are generally similar. These effects, whether acute or chronic, are thought to result from inhibition of cellular respiration and consequent histotoxic anoxia. However, the syndromes resulting from acute and chronic cyanide poisoning are distinctly different.

The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure. The LC_{50} or LD_{50} (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 ppm hydrogen cyanide causes death within one minute (ICMI, 2006). The LD_{50} for ingestion is 50-200 milligrams, or 1-3 milligrams per kilogram of body weight, calculated as hydrogen cyanide. For contact with unbraided skin, the LD_{50} is 100 milligrams (as hydrogen cyanide) per kilogram of body weight (ICMI, 2006).

2.8.1 MECHANISM OF CYANIDE TOXICITY

Cyanide has a high affinity for certain sulfur compounds (sulfanes, which contain two covalently bonded but unequally charged sulfur atoms) and for certain metallic complexes, particularly those containing cobalt and the trivalent form of iron (Fe^{3+})(MassDep, 1992). The cyanide ion can rapidly combine with iron in cytochrome a_3 (a component of the cytochrome aa_3 or cytochrome oxidase complex in mitochrondria) to inhibit this enzyme, thus preventing intracellular oxygen utilization(MassDep, 1992).The cell then utilizes anaerobic metabolism, creating excess lactic acid and a metabolic acidosis(MassDep, 1992). Cyanide also has a high affinity for the ferric iron of methaemoglobin and one therapeutic strategem induces the formation of methaemoglobin to which cyanide preferentially binds(MassDep, 1992).

The small quantity of cyanide always present in human tissues is metabolized at the approximate rate of 17 Fg/kg"min, primarily by the hepatic enzyme rhodanese, which catalyzes the irreversible reaction of cyanide and a sulfane to produce thiocyanate, a relatively nontoxic compound excreted in the urine(MassDep, 1992). (An elevated concentration of thiocyanate in either blood or urine is evidence of cyanide exposure). The limiting factor under normal conditions is the availability of a sulfane as a substrate for rhodanese, and sulfur is administered therapeutically as sodium thiosulfate to

accelerate this reaction. Because of the ability of the body to detoxify small amounts of cyanide via the rhodanese-catalyzed reaction with sulfane, the lethal dose of cyanide is time dependent; that is, a given amount of cyanide absorbed slowly may cause no biological effects even though the same amount administered over a very short period of time may be lethal(MassDep, 1992). In contrast, the LCt_{50} of each of the other chemical agents, which are not metabolized to the same extent as is cyanide, is relatively constant over time, and a lethal amount causes death whether administered within minutes or over hours(MassDep, 1992). The lethal dose for an adult, depends on the body weight and nutritional status. 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.

In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate (SCN). This detoxification requires sulphur donors that are provided by sulphur-containing dietary amino acids, cysteine and methionine (Okigbo, 1999). In subjects who have an adequate protein component of their diet, excess cysteine and methionine arenot required for protein synthesis and are degraded to inorganic sulphate and excreted.

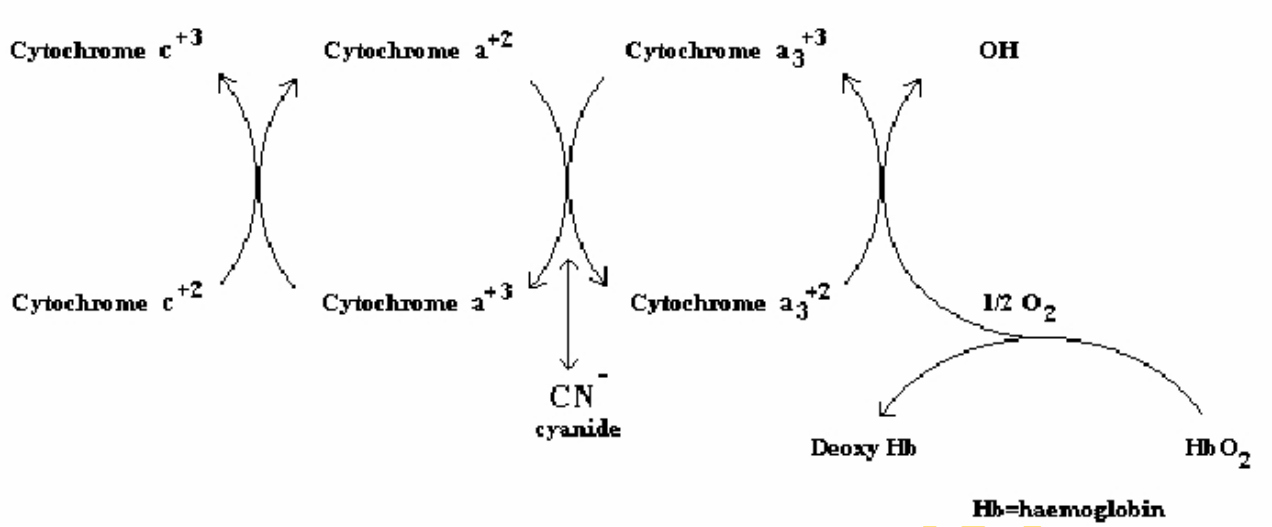


Figure 2.4. Inhibitive Binding to Cytochrome a-a3

2.8.2 POTENTIAL HEALTH EFFECTS IN HUMANS

2.8.2.1 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(Bhattacharya,2000). 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, 1987).

A characteristic sign of cyanide poisoning is the bright red colour of blood, which may result in red skin colour (Gosselin *et al*., 1984). There are many reports of cyanide

poisoning from accidental, suicidal and homicidal exposure to HCN or its salts (most commonly potassium or sodium cyanide). The majority of people who survive short-term cyanide poisoning do not have long-lasting effects. However, depending on the degree of exposure, there may be enduring effects from low oxygen, including impaired memory and mathematical abilities, personality changes, and altered control and coordination of movement (Hall *et al*., 1986).

Skin Contact:

Potassium cyanide is very toxic if absorbed through the skin. Skin contact with potassium cyanide solutions can cause symptoms similar to those described under "Inhalation" above. Potassium cyanide solutions are expected to be corrosive, based on pH. Corrosive materials 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 *et al*., 1985). In humans, the average lethal dose of hydrogen cyanide is estimated to be 60-90 mg (Gosselin *et al*., 1984). A few cases of Parkinsonism (a syndrome characterized by decreased mobility, muscular rigidity, and tremor) have

been reported in survivors of acute cyanide poisoning(Grandas *et al*., 1989). Ingestion is not a typical route for occupational exposure. If the hydrogen cyanide exceeds the limit an individual is able to detoxify/tolerate, death may occur due to cyanide poisoning. The acute oral lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg bodyweight. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man. Data on the oral lethal dose of cyanide for man in four cases of suicide, calculated from the amount of hydrogen cyanide absorbed in the body at the time of death, and from the amount of hydrogen cyanide found in the digestive tract, differed considerably and corresponded to doses of 0.58-22 mg/kg body weight (WHO, 1965).Acute cassava poisoning,which has led to the death of whole families, has been occasionally reported after the consumption of inadequately processed cassava (Osuntokun, 1981; Cliff & Countinho, 1995).

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.

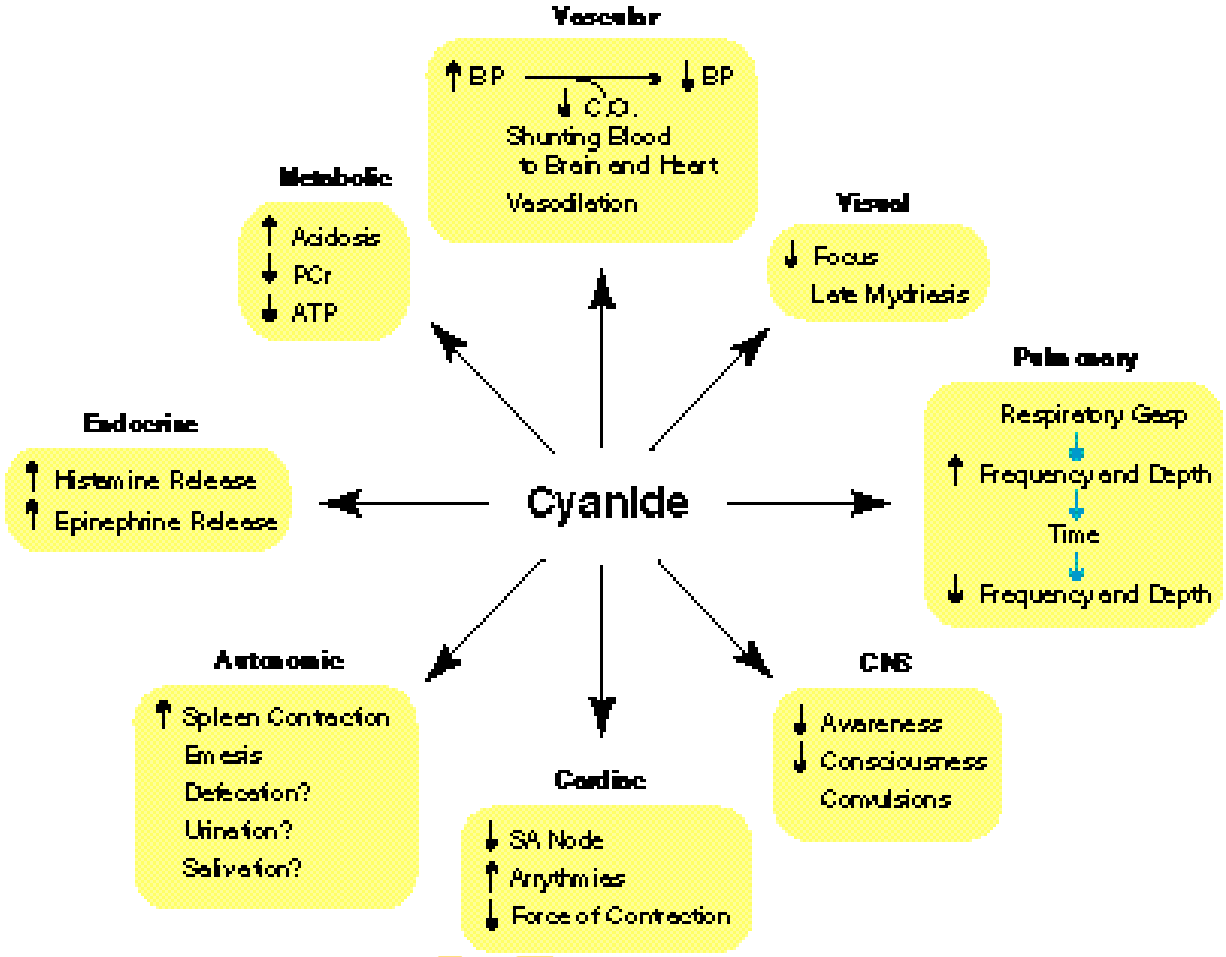


Figure 2.5: Cyanide toxicity pathways

Source: Baskin *et al*. 1998

*PCr: phosphocreatine

*ATP: adenosine triphosphate

*C.O.: cardiac output

2.8.2.2 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 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*., 1992).

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

1997). However, this diet is also high in scopoletin, a coumarin compound, which is believed to be responsible for some of the neurotoxic effects (Obidoa and Obasi, 1991).

Lungs/Respiratory System:

Two limited studies suggest that long-term cyanide exposure may be associated with laboured breathing. An increased incidence of effort-induced, laboured breathing was observed in 36 non-smoking male employees exposed for 5-15 years to 4.2-12.4 ppm cyanide from electroplating baths containing sodium and copper cyanide(El Gawabi *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 Gawabi *et al*., 1975).

Eyes/Vision:

Eye irritation was reported in 3 limited studies involving electroplating workers. Exposures, when specified, ranged from 4.2-15 ppm cyanide (Kumar *et al*., 1992). However, it is not possible to draw any specific conclusions about the eye irritation potential of long-term cyanide exposure, because electroplating workers are exposed to many chemicals that are irritating to the eyes (ATSDR, 1997). Degeneration of the optic

nerve and part of the retina (the macula) is found in people living in tropical areas with a diet high in cassava, a root rich in cyanogenic glycosides (Wilson, 1987). In some cases, these effects have been attributed to 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*., 1992) Statistical analysis of the results was not conducted. Blood chemistry changes (increased hemoglobin and lymphocyte counts and red blood cell damage) were observed in 36 non-smoking male employees exposed for 5-15 years to 4.2-12.4 ppm cyanide during electroplating operations (El Gawabi *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 (goitre). 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 Gawabi *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 *et al*., 1985).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 laboratory rodents or dogs(Diasolua-Ngudi, 2005). 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(Diasolua-Ngudi, 2005).

Health Comments:

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

2.8.2.3 Long-Term Studies and Cyanide Diseases

Konzo

'Konzo' is a local Zairean term for a disease first described in 1938 in the Democratic Republic of Congo (formally Zaire) by Trolli (1938), but has also been observed in Mozambique, Tanzania, Central African Republic and Cameroon (Ministry of Health, Mozambique, 1984a; Howlett *et al*., 1990; Tylleskar *et al*., 1992, 1994; Lantum, 1998; Ernesto *et al*., 2002). Konzo is an upper motor neuron disease characterised by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age. Severe cases have a spastic toe-scissor gait or patients will not be able to walk at all, and the arms and speech may also be affected. A long-term follow-up of konzo patients showed that the neurological signs

in konzo patients remained constant; however, functional improvement may occur (Cliff*et al*., 1997). High urinary thiocyanate concentrations and presence of ankle clonus were 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 (reviewed by Rosling, 1987).

2.9 TREATMENTS 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, 1984; Isomand Borowitz, 1995). Their mechanism of action, efficacy and toxicity have been reviewed as part of a joint IPCS (UNEP, ILO, WHO)/CEC project

to evaluate antidotes used in the treatment of cyanide poisoning (van Heijst andMeredith, 1990). A wide variety of compounds have been used as cyanide antidotes and they have been classified into four major groups based on their mechanism of action: Scavengers, Detoxification, Physiological and Biochemical (Isom and Borowitz, 1995).

2.9.1 SCAVENGERS

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

a. Methaemoglobin 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 methaemoglobin to complex cyanide. Cyanide preferentially competes with the Fe+++ of methaemoglobin as compared to that of cytochrome oxidase, and eventually binds with the former to form cyanmethaemoglobin (Way, 1984; Jandorf andBodansky, 1946). Thereby, the activity of inhibited cytochrome oxidase is restored. The various methaemoglobin 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 (Way, 1984; van Heijst and Meredith, 1990; van Heijst *et al*., 1987). However, the efficacy of amyl nitrite as methaemoglobin inducer remained disputed on account of its inability to generate methaemoglobin greater than 6% (Bastian and Mercker, 1959), while about 15% is required to challenge one LD_{50} 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 methaemoglobinaemia (Vick and Froehlich, 1985).

(ii) Sodium nitrite:

Sodium nitrite (SN) is the most prevalent drug of choice for cyanide poisoning (Way, 1984; Chen *et al*. 1952). When given intravenously, it takes about 12 min to generate approximately 40% of methaemoglobin (van Heijst *et al*., 1987). In spite of this delay in inducing a significant level of methaemoglobinaemia, a reasonable protection offered by SN can be ascribed to its vasodilatory property (van Heijst and Meredith, 1990). A

serious drawback with SN is that intravenousadministration may be accompanied by serious cardiovascular embarrassment, particularly in children, for whom an adjusted dose is recommended (Berlin, 1970). Since SN induced methaemoglobinaemia 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 and Meredith, 1990).

(iii) 4 - Dimethylaminophenol:

The relatively slow rate of methaemoglobin formation by SN prompted the development of rapid methaemoglobin formers like aminophenols (Bhattacharya, 2000).The treatment of choice for cyanide poisoning in Germany is 4-dimethylaminophenol (DMAP) (Bhattacharya, 2000). A dose of 3.25 mg/kgintravenous injection of DMAP was reported to produce methaemoglobin level of 30% within 10 min and 15% methaemoglobinaemia was attained within one minute without any immediate effect on cardiovascular system (Kiese and Weger, 1969). However, there are differences in individual susceptibility to DMAP, which may result in an undesirable level of methaemoglobinaemia even after normal therapeutic doses (van Dijk *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 methaemoglobin 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 methaemoglobinaemia provided sustained prophylaxis in rats (Bhattacharya *et al*., 1991).

(iv) Other methaemoglobin formers:

Hydroxylamine (HA) was yet another rapid methaemoglobin inducer (Kruszyna *et al*., 1982) 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*., 1993) 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 methaemoglobin 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 methaemoglobin formers and directly sequestered cyanide to protect a 4 X LD_{90} 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; intravenous administration) 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 and Meredith, 1990).

(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. Recorded side effects of hydroxocobalamin include 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, 1984). Sodium pyruvate was reported to effectively challenge acute cyanide poisoning in mice (Schwartz *et al*., 1979). 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 (Hume*et al*., 1996). 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).

2.9.2 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 (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 intravenously, 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 and Meredith, 1990). Endogenous augmentation of Rhodanese has not been worked out extensively but exogenous supplementation has been reported to accelerate the transulfuration of cyanide to thiocyanate (Bhatt and Linnell, 1987). However, stability and sensitivity of the enzyme remains to be addressed.

2.9.3 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, 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).

2.9.4 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, 1984). Its protective effect was attributed to its α- adrenergic blocking property (Kong *et al*., 1983). Subsequently, the antidotal activity of chlorpromazine was related to its ability to sustain cellular calcium homeostasis and maintenance of membrane integrity by preventing peroxidation of membrane lipids (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. (Nikhand *et al*., 1994).

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).

2.9.5 PROBLEMS ASSOCIATED EXISTING ANTIDOTES

Numerous antidotes are available but their safety and efficiency has been questioned. Oxygen counteracts efficiently cyanide action at the mitochondrial level(Mégarbane*et al*., 2003). Sodium thiosulfate, methaemoglobin forming agents and cobalt compounds act efficiently by complexing or transforming cyanide into non-toxic stable derivatives. However, regarding the main clinical condition of cyanide poisoning, i.e. smoke inhalation, the efficiency of antidotes should not only be taken into account but also their safety. Sodium thiosulfate is both efficient and safe, but acts with delay(Mégarbane*et al*., 2003). Methaemoglobin-forming agents are potent, but due to the transformation of hemoglobin into methaemoglobin, they impair tissue delivery of oxygen(Mégarbane*et al*., 2003). Experimental data showed increased mortality in carbon monoxide- and cyanide-poisoned rats treated with these agents. Cobalt EDTA and hydroxocobalamin are efficient and act immediately. Cobalt EDTA is more potent on a molar basis; however, numerous side effects limit its use to evidenced cyanide poisoning(Mégarbane*et al*., 2003). In a prospective study, hydroxocobalamin appeared safe in fire victims with or without cyanide poisoning. The only reported side effect was a red coloration of skin and urine (Mégarbane*et al*., 2003).

2.10 LEAFY VEGETABLES

Vegetables are succulent plants grown mainly ingardens and consumed as a side dish/soup with starchystaples, especially among some Nigerian tribes. They areof special nutritional importance being sources of fat andwater soluble vitamins (ß-carotene, ascorbic acid riboflavin, thiamine and niacin), minerals and fibre. Theyincrease variety, add flavour and zest to diets andconstitute important common foodstuff and component ofmost meals in Nigeria. They form concentrated source ofnutrients in the home diet and are used as foodsupplement. In tropical Africa countries, Nigeria inclusive,vegetables are the cheapest and most valuable source ofimportant proteins, vitamins and essential amino acids(Guarino, 1995).

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 (Aletorand Adeogun, 1995). Leafy vegetables are important items of diet in many Nigerian homes. According to Oke (1966) vegetables, about 5.0g dry materials per meal are taken per family twice a day in southeastern Nigeria. With the rapid rate of over 1.4 percent population growth rate in Nigeria (Madu,

2001); most families presently take vegetables three times per day. 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 valuable source of important 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, 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 (Mosha and Gaga, 1999). Theyadd 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 some 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.

2.10.1 CULTURAL ASPECTS IN THE CONSUMPTION OF FOREST FOODS

Plant foods especially vegetables contribute substantially to both local diets and ethno medicine in developing countries especially Nigeria (Okafor, 1980; Gbile and Adesina, 1986).Edible plant or animal species in a given area may or not be eaten, depending on the local culture. The consumption of foods is certainly as much a social issue as a biological one. Food is an essential part of most social interactions and rites. The selection of foods must be understood in the context of the social, political and economic processes that underlie them. Individual decisions regarding food acquisition and consumption are seldom independently made or value-free(FAO, 1989). They are generally guided by local cultural perceptions, attitudes and beliefs(FAO. 1989).

Wild foods often have a cultural value and are consumed during special feasts. Their taste is often considered superior by local populations(FAO. 1989). Some forest sites and/or species may also have a sacred valuewhich varies from one village to another(FAO. 1989). However, traditions are continuously changing according to new perceived opportunities. Thisevolution is accelerated by changes in attitude in the younger generations and immigration of people with different values. Foods which once provided security against famine following hurricanes and droughts have now been replaced by more recently introduced cassava and sweet potato and have become obsolete as famine foods(Olsson, 1991). However, these foods retain a high cultural value which cassava and sweet potato have not acquired even though they provide food security. They therefore do not run the risk of being depleted from ritual feasting(Olsson, 1991). Local perceptions of the value of a given food are generally independent from its nutritional content (FAO. 1989). In any society, the use of foods is determined by a series of unwritten rules and codes (FAO. 1989). Taboos and ritually marked foods may, for example, determine the selection of foods for specific social groups (e.g. women, children, adult males). These are often linked to local health beliefs. Many foods in particular spices are considered to have properties that improve health and are therefore used as self-administered medication (FAO. 1989).

Plate2.1:*Gnetum africanum* plant

2.10.1.1*GNETUM AFRICANUM*

Gnetum africanum is one of the most popular green leafy vegetable in Nigeria and is gaining equal popularity as a delicious food leaf in other African countries such as Cameroon, Gabon, Congo and Angola (Eyo and Abel, 1983). *G.africanum* leaves are

widely consumed in the South Eastern Nigeria due to its palatability and taste. It is now eaten as a vegetable salad when mixed with palm oil. The popularly known afang soup that is often listed in many continental restaurant menu, is prepared from these leaves which sometimes is cooked with waterleaves (*Talinum triangulare*) to give the soup a special savour.

Gnetum africanum (afang) grows as a wild evergreen climbing plant in the rainforest of Nigeria where it is searched for and highly priced in the regional markets. It is recently being cultivated in South Eastern Nigerian homes as exotic plants. It belongs to the family *Gnetacea* and the order *Gnetales* (Dutta, 1981). Common names for the plant includeokazi, afang, eru, koko, nkoko,and wild spinach.Available literature reveals that both the leaf and the seed in particular have shown medicinal efficacy in the treatment of enlarged spleen, sore throats, reduction of pains of child-birth, antidotes to some forms of poison and snake bite(Ekop, 2007).The mineral element content, amino acid content and proximate composition of the leaves has been reported by Eyo and Abel (1983).

Botanic Description

Dioecious liana up to 10 m long but sometimes longer; branches somewhat thickened at the nodes, glabrous. Leaves decussately opposite, sometimes in whorls of 3, simple; stipules absent; petiole up to 1 cm long, canaliculate above; blade ovate-oblong to elliptical-oblong, rarely lanceolate, 5–14 cm × 2–5 cm, base attenuate, apex abruptly acuminate, obtuse or minutely apiculate, entire, thick-papery, glabrous, pale green above, paler beneath, with 3–6 pairs of strongly curved lateral veins looped near the margin. Inflorescence an unbranched catkin, axillary or terminal on a short branch, solitary but male inflorescences at apex of branches often in groups of 3, up to 8 cm long, jointed, peduncle 1–1.5 cm long, with a pair of scale-like, triangular bracts; male inflorescence with slender internodes and whorls of flowers at nodes; female inflorescence with slightly turbinate internodes and 2–3 flowers at each node. Flowers are small, 2 mm long, with moniliform hairs at base and an envelope; male flowers with a tubular envelope and exserted staminal column bearing 2 anthers; female flowers with cupular envelope and naked, sessile ovule. Seed resembling a drupe, ellipsoid, 10–15 mm × 4–8 mm, apiculate, enclosed in the fleshy envelope, orange-red when ripe, with copious endosperm. In Africa, there are only two species, *G.africanum* and*G.buchholzianum*. The specific epithet africanum refers to its African origin. The plant is threatened with disappearance

because of intensive gathering and cultural practices which are destroying the forests which support these plants. Possible introduction into farm systems is a step in the right direction in conserving this plant.

Other botanical information

Gnetum comprises approximately 35 species of small trees, shrubs or most often lianas, found in tropical South and Central America (about 7 species), Africa (2 species) and Asia (about 25 species)(Schippers and Besong, 2004). They look much like dicotyledonous flowering plants (having opposite leaves with a net venation and cherry-like seeds), although in fact they are gymnosperms (Schippers and Besong, 2004). The 2 African species, which are very similar, have been classified in section *Gnetum*, subsection *Micrognemones*(Schippers and Besong, 2004). *Gnetum africanum* has leaves which are relatively thin and pale green. Its male catkins have slender internodes of equal width from the base to the tip. *Gnetum buchholzianum* has thick dark green leaves. The male catkins have thick internodes widening towards the terminal part (Schippers and Besong, 2004).

Ecology and distribution

Wild spinach can be found in rainforest from sea-level up to 1200 m altitude, and prefers an annual rainfall of about 3000 mm(Schippers and Besong, 2004). It is usually found with other climbers on middle- and under-storey trees, frequently forming thickets. It can also be found in riverine forest in areas that are otherwise too dry for the species. *Gnetum africanum* is mostly found at the periphery of primary forest and in secondary forest(Schippers and Besong, 2004). Today, it is more common than *Gnetumbuchholzianum*, which is mainly found in primary forest, especially near openings created by fallen trees(Schippers and Besong, 2004).

(i.) Natural Habitat

Gnetum africanum is an endangered liane normally found in humid tropical forest understoreys. It extends in distribution from South East Nigeria, to Congo and as far as Angola in the south.

(ii) Geographic distribution

Native: Angola, Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Gabon, Nigeria.

(iii) Reproductive Biology

This is a dioecious plant with distinct differences in male and female inflorescence structure and size.

Growth and development

Both African *Gnetum* species are lianas with two different types of stems. The orthotropic ones have small, scale-like leaves and rapidly grow vertically, reaching the main branches of a tree where they produce plagiotropic stems with fully developed leaves. The orthotropic stem continues climbing until it reaches the canopy where it branches into several leafy stems. Female plants often show more vigorous growth with stronger stems than male plants. This is more obvious in *Gnetum africanum* than in *Gnetum buchholzianum*.

Wild spinach continues to grow during the dry season and new shoots may develop where the stem has been cut or where side shoots have been removed. New shoots are also formed from rhizomes that spread along the forest floor. The distinctly coloured drupe-like seeds are probably dispersed by birds and other animals.

Propagation and planting

Experimental plantings for domestication are being made with both species. Nurseries are now concentrating their efforts on *Gnetum buchholzianum* because it is preferred by traders and is more vigorous. Moreover, male vines of *Gnetum africanum* are less appreciated because of their smaller, thinner and paler leaves, and because of their less vigorous growth. For *Gnetum buchholzianum* there is no need to harvest only female plants. However, the field trials might show that *Gnetum buchholzianum* is more difficult to cultivate than *Gnetum africanum* because the former probably requires more shade than the latter(Schippers and Besong, 2004). In experiments in Cameroon, propagation by seed was difficult because the seed is reluctant, germination taking one year or more. It is assumed that seeds need pretreatment, such as passing through the intestines of a bird, fruit bat, squirrel or other animal, before they germinate(Schippers and Besong, 2004).

Seed is normally found only in the tree canopy. Seed collection is thus far from easy, a further reason why wild spinach is hardly cultivated(Schippers and Besong, 2004).

Methods of vegetative propagation using leafy stem cuttings have recently been developed. It is recommended that leaf blades of cuttings be trimmed in half. Nursery beds under shadeand made of well-decomposed sawdust or fine river sand can be used for propagation. Ectomycorrhizae assist the roots in absorption of nutrients; the most common species reported is *Scleroderma sinnamarense*. After about 6 weeks, the rooted cuttings are transferred to polythene sleeves, bamboo pots or other containers where they remain for a further 2–3 months. The soil mixture for these containers consists of 25% sand and some compost, supplemented with forest soil. Field planting, preferably next to a young tree or shrub, takes place at the beginning of the rainy season.

Production and international trade

In trade, consignments of *Gnetum africanum* and *Gnetum buchholzianum* are often mixed. Traders will pay more for the thick dark green leaves of the latter, but much variation is also caused by growing conditions. Most wild spinach is consumed locally, but intensive trade has developed from Cameroon and more recently also from Gabon and the Central African Republic to meet the large demand in Nigeria. Most wild spinach from Cameroon, Gabon and the Central African Republic is transported to Idenau, a coastal village in Cameroon, and from there by boat to Nigeria. Estimates for the annual export of afang leaves (both species) to Nigeriarange between 2500t and 4000t(Schippers and Besong, 2004). Another major marketing centre is the Koilo Region in Congo. Other marketing centres in Cameroon are Campo near Kribi for export to Gabon and the Mfoundi market in Yaoundé. Dried shredded leaves are exported, mainly from Nigeria to the United States and to a lesser extent from other countries to France and the United Kingdom (Schippers and Besong, 2004).

Adulterations and substitutes

The leaves of *Gnetum africanum* can be replaced by those of the other wild spinach species, *Gnetum buchholzianum*, or leaves of the shrub *Lasianthera africana*, P.Beauv., which impart a similar taste to the dish (Schippers and Besong, 2004).

Properties

The composition of *Gnetum africanum* leaves is probably comparable to *Gnetum buchholzianum.* The dry matter content of fresh leaves is much higher than for other dark or medium green leaf vegetables. This gives a feeling of firmness during preparation; hence certain consumers consider wild spinach as a substitute for meat. The leaves of *Gnetum africanum* are somewhat thinner and paler than the dark green leaves of *Gnetum buchholzianum*. Consequently, the content of micronutrients in the latter might be somewhat higher. *Gnetum africanum* constitutes a significant source of protein, particularly essential amino acids, and mineral elements. *Gnetum africanum*is a significant source of protein (16.5% dry wt.) carbohydrates (70.6% dry wt.), essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), non essential amino acids (aspartic acid, serine, glutamic acid, proline,glycine, alanine, cysteine, tyrosine histidine and arginine) and mineral constituents i.e. macro and micro-elements (7.0% dry wt.) (AFTDatabase, 2004). The leaf fat content in gnetum is significant, up to 14.20% (Okafor *et al.*, 1996). Wild spinach leaves contain C-glycosylflavones, including 2"-xylosylisoswertisin and 2"-glucosylisoswertisin, compounds that are only known from these two species; characteristic of *Gnetum africanum* is the presence of 2"-O-rhamnoylisoswertisin and apigenin-7-hesperidoside and the absence of vitexin and 2"-O-glycosylvitexin.

Table 2.2: Chemical composition and nutritional value of *Gnetum africanum*

Constituents (%)	*Gnetum Africanum*
Moisture	7.60 ± 0.01
Ash	8.00 ± 0.02
Crude lipid	7.10 ± 0.02
Total carbohydrate	30.38 ± 0.01
Crude protein	19.67 ± 0.01
Crude fibre	27.25 ± 0.00
Sodium	10.50± 0.00
Potassium	3.40± 0.00
Calcium	13.60± 0.00
Magnesium	3.60± 0.00
Iron	78.75± 0.00
Phosphorus	0.35± 0.00
Cyanogenic glycoside	1.73 ± 0.01

Source: Iheanacho and Udebuani (2009)

Table 2.3: Qualitative phytochemical constituents of leaves of*Gnetum africanum*

Substances	Presence
Phenolic substances	++
Flavonoids	++
Anthocyanidins	+++
Phytosterols	+
Tannins	++
Saponins	++
Alkaloids	+
Glycosides	++
Cyanogenic glycosides	++
Cardiac glycosides	++

+: Presence

Source: Iweala *et al*. (2009)

Uses

Fresh leaves of *Gnetum africanum* and the very similar *Gnetum buchholzianum*, in English, both called wild spinach (koko in French), are widely used as a vegetable, as are those of other American and Asian *Gnetum* species. *G. africanum* holds an important place in the diets of many people in central Africa (Schippers and Besong, 2004). In the Congo, Gnetum consumption has been evaluated at 2g /capita. They are usually cooked with meat or fish and occasionally consumed as a salad. Leaves are shredded into thin strips and are often eaten as part of a mixture in, for example, a groundnut-based stew (Schippers and Besong, 2004). To soften this rather tough vegetable, people often mix it with waterleaf (*Talinum triangulare* Jacq.). Shredded leaves can be dried and preserved for later use. Women play a great role in the gathering and selling of the much relished leaves all year round. Commerce in Gnetum has increased considerably. The seeds are eaten in Cameroon and DR Congo (Schippers and Besong, 2004).

In Nigeria, wild spinach is used for treatment of piles and high blood pressure and also as medicine against enlarged spleen, sore throat and as a purgative. In the Central African Republic, the leaves are eaten to treat nausea and as an antidote to arrow poison made from *Periploca nigrescens* Afzel (Schippers and Besong, 2004). West Africa chopped leaves are used as a dressing on furuncles to hasten maturation. The stem is used in making preparations to ease childbirth. In Cameroon, the leaves are chewed to mitigate the effects of drunkenness and they are taken as an enema against constipation and to ease

childbirth. They are also used to treat boils and fungal infections on the fingers. The supple stem is sometimes used in making traps for game catching and porterage straps (Schippers and Besong, 2004).

Services

Gnetum africanum has significant cultural value in traditional ceremonies.Traditionally, gnetum consumption was limited to localized population groups. With the recent demographic mix, it has spread to the population at large and gnetum has become a socio-cultural symbol. Many restaurants now offer specialities including gnetum in the sub-region and it is even exported to Europe (Schippers and Besong, 2004). With an average daily consumption of 2 g per day in Congo, this wild species is now threatened with extinction (Schippers and Besong, 2004).

Management

Wild spinach is still mainly collected from wild stands, but farmers often retain it when clearing fields. If cultivated, farmers need to provide support, e.g. by using commercial plantations of rubber trees, oil palm and other tree crops. Fences were only found to be successful when there is enough shade, and they are generally too expensive. Fully exposed plants do not grow well; their leaves are thin and pale green, and traders reject them. In experiments, nutrients, especially nitrogen, have shown a positive effect on growth and rate of leaf development(Schippers and Besong, 2004).

Diseases and pests

Mealy bugs are the main pest in the nursery. When wild spinach is grown along dead poles attacked by termites, these insects will damage adjacent leaves. Diseases have not been found to reduce productivity of wild spinach (Schippers and Besong, 2004).

Harvesting

The current method of harvesting, especially for export trade, is to pull the stems or branches from trees. This leads to large-scale destruction of natural stands. Occasionally, trees have to be cut to reach leafy stems in the canopy. This is mainly done during the dry season when the forest is more accessible and when there is little work on the farm. Controlled harvesting, in which only side shoots or parts of stems are collected, is clearly better than destructive harvesting. After controlled harvesting, new shoots may develop

where a stem has been cut or where side shoots have been removed. Preliminary observations indicate that 3–4 harvests per year are possible, still allowing for substantial regrowth. More frequent harvesting will result in thin leaves that are considered inferior. The first harvest may take place 6–9 months after planting. The total lifespan of wild spinash is estimated at over 10 years(Schippers and Besong, 2004).

Yield

Preliminary observation indicates that in cultivation during the first harvest year, the fresh leaf yield may reach 20 t/ha. This may double in subsequent years(Schippers and Besong, 2004).

Handling after harvest

The leafy stems of gnetum remain fresh for at least a week. Stems collected from the forest are brought to collecting points from where they are either sold in the local market or exported. For this trade, whole leafy stems are packed in large bales. Selection takes place for size and texture of the leaves, and is mainly determined by species. *Gnetum buchholzianum* is more popular with consumers and more expensive because its leaves are generally thicker than those of *Gnetum africanum*. Leaves are shredded before consumption or prior to drying(Schippers and Besong, 2004).

Genetic resources

Wild spinach is hardly cultivated at all at present, but there is massive exploitation of the remaining natural stands, which have almost disappeared in Nigeria and are becoming scarce in Cameroon, Gabon and the Central African Republic. There is an urgent need to collect and preserve the diversity found within the two African *Gnetum* species, preferably throughout their natural range. Accessions need to be evaluated for their agronomic potential and for their ability to germinate without the need for interventions. A small collection is currently held at the Limbe Botanic Garden, Limbe, Cameroon(Schippers and Besong, 2004).

Plate 2.2**:** *Ocimum gratissimum*plant

2.10.1.2 *OCIMUM GRATISSIMUM*

Ocimum gratissimum (Linn) of the family Lamiacea is an aromatic perennial plantwidely distributed in the tropical and warm regions of the world (Okigbo and Ogbonna, 2006).It is commonly called scent leaf in English.In India, it is called Tulsi (Ahmed *et al*., 2002). In the southern part of Nigeria, the plant is called "effinrin-nia" by the Yoruba-speaking tribe, "Nchoanwu" in Igbo, while in the northern part of Nigeria; the Hausas call it "Daidoya" (Effraim *et al*., 2000).In Nigeria, it is found in savannah and coastal areas.

Botanical Description

It is a perennial plant that is woody at the base. It is an erect, multi-branched perennial shrub that grows up to a height of two meters with a taproot and many adventitious side rootlets. The leaves are simple, opposite or whorled with several oil glands and possess a peculiar scent smell due to its composition of volatile essential oils. The leaves are broad

and narrowly ovate, usually 5-13cm long and 3-9cm wide. It is a scented shrub with lime-green fuzzy leaves. It produces an inflorescence that is capitate and reduces apical dominance while increasing branching. The flowers are zygomorphic, bilobal and bisexual with five petals and sepals and four stamens. The gynecium has two carpels ascending from the ovary. The fruit has a group of four nutlets each with a brown seed that has scanty or no endosperm.

Ecology and distribution

The perennial plant *Ocimum gratissimum* is commonly found in the tropical Asia, especially India and in some other places including West Africa especially, Nigeria. It is the most abundant of the genus *Ocimum* (Efiri, 2009).

Chemical Constituents:

Leaves of *O. gratissimum* have been found rich in alkaloid, tannis, phytates, flavonoids, oligosaccharides, and has tolerable cyanogenic content (Ijeh *et al*., 2004). Its leaves also contain methylchaylcol, linalol, eugenol, thymol and xanthamicrol and the amount produced is dependent on the area that it is cultivated as well as part of the plant (Okujagu *et al*., 2005;Odebiyi and Sofowora, 1978). Its other constituents include saponins, steroids, camphor, estragol, litral, anethol, hydrocynamate and terpenes.

Traditional Uses

Ocimum gratissimum has both culinary and medicinal uses (Iwu, 1986). It is mainly used as a spice to flavor foods and meats (Okigbo, 1977). Its use as spice is known to reduce microbial load and extend the shelf life of foods. The components of *O. gratissimum* has biological activity such as antidiabetic, antiseptic, antitussive, antihelmintic, antispasmodic and antimicrobial (Gbolade, 2009; Akinyemi *et al*., 2004; Lopez *et al*., 2005). The leaves of *O. gratissimum* are used as a laxative and its infusion serves as a relief for respiratory disorders, headaches, fever, diarrhea, dysentery, pile and convulsion (Idu *et al*., 2005; Danziel, 1980).

The whole plant is used as a remedy for gonorrhea, catarrh conditions, cough, constipation, ringworm, flatulence, and hypertension (Odugbemi and Akinsulire, 2006). Its leaves are used as sponge to remove skin blemishes and have been formulated into skin creams used for treating dermatological disorders as well as toothpastes used in maintaining oral hygiene (Odebiyi and Sofowora, 1978). The mucilaginous nutlets are

used for treatment of cough and mixed with a drink used against gonorrhea and intestinal disturbance. The Igbo name of *O. gratissimum*'Nchoanwu' literally means mosquito-repellant and thus, its presence around the home is believed to repel mosquitoes as a basis for the prevention of malaria. The extracts of the leaves are applied externally in treatment of conjunctivitis, rheumatic pain, dressing of wounds and lumbargo. The hypoglycaemic activity of the plant has also been reported (Aguiyi *et al*., 2000).

Services

Boundary or barrier or support: *O. gratissimum*is also cultivated as a hedge plant.

Table 2.4: Qualitative phytochemical constituents of leaves of *Ocimum gratissimum*

Substances	Presence
Phenolic substances	+
Flavonoids	+
Anthocyanidins	+
Phytosterols	+
Tannins	+++
Saponins	++
Alkaloids	ND
Glycosides	++
Cyanogenic glycosides	+
Cardiac glycosides	++

+: Presence, ND: Not Detected

Source: Iweala and Obidoa (2010)

Table 2.5: Chemical composition and nutritional value of *Ocimum gratissimum*

Constituents (mg/100g)	*Ocimum gratissimum*
Ash	10.25
Lipids	13.7
Carbohydrates	2.38
Proteins	11.4
Pyridoxine	0.41
cysteine	1.60
Casein hydrolysate	89.99
Glycine	3.82
Fibres	9.26
Thiamine	0.20
Ascorbic acid	15.35
Nicotinamide	0.86
Total oxalate	0.86
Hydrocyanic acid	0.51
Acids	12.25
Calcium	64.80
Magnesium	84.10
Iron	13.36
Zinc	6.85
Copper	5.69
Sodium	8.2
Potassium	86.24
Iodine value	34.25

Source:Alabi *et al*., 2005

2.10.2LYOPHYLIZATION

This is a method of drying food or blood plasma or pharmaceuticals or tissue without destroying their physical structure; material is frozen and then warmed in a vacuum so that the ice sublimes.

Principle of Lyophylization (Freeze Drying)

Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. There are however, many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books and other items damaged by water and the concentration and recovery of reaction products.Freeze drying involves the removal of water and other solvents from a frozen product by a process called sublimation. Sublimation occurs where a frozen 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.

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, that is more difficult to freeze dry. Slower cooling results in larger ice crystals with less restructive channels in the matrix during the drying process. Products freeze in two ways depending on the make up of the product. The majority of the product that is subjected to freeze drying consists primarily of water. Most samples that are to be freeze dried are eutectics which are a mixture of substances that freeze at lower temperatures than the surrounding water. When the aqueous suspension is cooled, changes occur in the solute concentrations of the product matrix. As cooling proceeds, the water is separated from the solutes as it changes to ice, creating more concentrated areas of solute. This pocket of concentrated materials, have a

lower freezing temperature than the water. Although a product may appear to be frozen because of all the ice present, in actuality, it is not completely frozen until all of the solute in the suspension is frozen. The mixture of various concentrations of solutes, with the solvent constitutes the eutectics of the suspension. Only when all the eutectic mixture is frozen is the suspension properly frozen. This is called the eutectic temperature. It is very important in freeze drying to pre freeze the product to below the eutectic temperature before beginning the freeze drying process. Small pocket of unfrozen material remaining in the product expand and comprise the structural stability of the freeze dried product.

Primary Drying

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

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.

2.11 HAEMATOLOGICAL INDICES OF TISSUE DAMAGE AND TOXICITY

Blood is made up of a liquid portion and all the various blood cells. It functions to transport nutrients and oxygen to the cells; wastes and carbon dioxide to the organs responsible for their removal or breakdown; and also to defend the body against bacteria, viruses, and other organisms. The liquid portion of blood is referred to as plasma, if the blood was not allowed to clot, and serum, if it was. This liquid portion, without the cells, is generally a straw or light yellow color. The liquid portion of the blood is used in the chemistry tests.

2.11.1 Complete blood count

The complete blood count (CBC), sometimes referred to as a full blood examination orfull blood count (FBC), is a common blood test that evaluates the three major types of cells in the blood: red blood cells, white blood cells, and platelets. It can depict so much about health status. It is important for diagnosing conditions in which the number of blood cells is abnormally high or abnormally low, or the cells themselves are abnormal. The CBC can also test for loss of blood, abnormalities in the production or destruction of blood cells, acute and chronic infections, allergies, and problems with blood clotting. Every drop of blood literally contains millions of blood cells. Although the sample drawn for a CBC may seem small, it contains such huge numbers of cells that it is an excellent and accurate portrayal of the total numbers of these cells found in the bloodstream. The CBC is concerned with the quantities and types of red blood cells, white blood cells, and platelets.

Many conditions will result in increases or decreases in the cell populations. Some of these conditions may require treatment, while others will resolve on their own. Some diseases, such as cancer (and chemotherapy treatment), can affect bone marrow production of cells, increasing the production of one cell at the expense of others or decreasing overall cell production. Some medications can decrease WBC counts, and some vitamin and mineral deficiencies can cause anaemia. The CBC test may be ordered by the doctor on a regular basis to monitor these conditions and drug treatments.

2.11.1.1 Red blood cell tests

Red blood cells (RBC), which contain the molecule hemoglobin,areresponsible for carrying oxygen from the lungs to cells throughout the body. The oxygen taken into the

body attaches to the hemoglobin as the RBC pass through the lungs. The RBC then deliver the oxygen to all the other cells in the body and take the carbon dioxide back to the lungs to be exhaled. RBC are formed in the bone marrow. The bone marrow constantly produces new RBC, since the life span of an RBC is only about 120 days. The body can respond quickly to maintain the number of RBC present in the blood vessels. The body measures their numbers simply by evaluating the quantity of oxygen being supplied to its tissues. Insufficient amount of oxygen in the body, results in a need for more working RBC. The immediate need for more RBC leads to the production of more immature cells (called reticulocytes) which are released into the circulation from the bone marrow. However, the presence of adequate cellsslows down the release of new ones.
Red blood cellsare measured by three main tests. These include:

Red Blood Cell Count: This is an estimation of the number of red blood cells per litre of blood. Abnormally low numbers of red blood cells may indicate anaemia as a result of blood loss, bone marrow failure, malnutrition such as iron, folic acidand vitamin B_{12}deficiency, over-hydration, or mechanical damage to red blood cells. With anemia, the cells get insufficient oxygen to function normally. This is indicated by weak feelings and pale look.

Abnormally high numbers of red blood cells may indicate congenital heart disease, some lung diseases, dehydration, kidney disease or polycythaemia vera. Polycythemia(a condition for very high counts of RBC) may indicate that the red blood cells clump together and block tiny blood vessels (capillaries), resulting in difficulty of the red blood cells to carry oxygen.

Haemoglobin (Hb):Haemoglobin is an iron-containing compound that fills up the red blood cells and gives the blood cell its red color. Hb is a protein in red blood cells that actually transports oxygen from the lungs to the rest of the body. Hb test evaluate the RBC by quantifying the amount of hemoglobin present in the blood. It is a good measure of the blood's ability to carry oxygen throughout the body. Hb is expressed in grams per decilitre. Measuring the concentration of haemoglobin in the blood can help diagnose anaemia, a condition caused by a deficiency of haemoglobin. Anaemia can arise due to:

- inadequate production of red blood cells in the bone marrow;
- inadequate iron intake;

- inadequate folate or vitamin B_{12} intake;
- microscopic bleeding or other blood loss;
- blood cell destruction;
- a chronic illness; or
- a defect in the haemoglobin molecule itself.

This measurement may also detect abnormally high concentrations of haemoglobin. This may occur in people with chronic lung disease, as an adaptation to high altitudes, or because of an abnormal increase in red cell production by the bone marrow (polycythaemia vera).

Hematocrit (HCT):This is also referred to as the packed cell volume (PCV) which measures thefraction of the whole blood volumethat consists of the red blood cells. The value is given as a percentage of red blood cells in a volume of blood. For example, a hematocrit of 38 means that 38% of the blood's volume is made of red blood cells. Hematocrit and hemoglobin values are the two major tests that show if anemia or polycythemia is present.

In low PCV, there are fewer red cells in the body than is expected. This condition is referred to as anemia. In severe cases of anemia, the animal would probably have pale membranes in its mouth and seem weak and tired, since its body would be getting less oxygen than needed. Anemia is further classified as either regenerative or nonregenerative. In the former, even though the number of red blood cells is lower than normal, the body is responding by releasing new reticulocytes into the circulation. In the nonregenerative anemia, there is no or very few immature RBC in the sample and the body continues to lose red blood cells, but no new ones are produced. A nonregenerative anemia is very serious and will quickly become life-threatening. A low haematocrit may also indicate blood loss, bone marrow failure, leukaemia, multiple myeloma, nutritional deficiency, over-hydration or rheumatoid arthritis.

Elevated PCV may indicate dehydration (for example, due to burns or diarrhoea) usually seen in dehydrated animals as their blood is becoming more concentrated, eclampsia (a serious condition that can occur during pregnancy) or polycythaemia vera. It is noted in other conditions, such as some cases of shock, response to high altitudes (the air is 'thinner,' therefore there is less oxygen, so more RBC are put into circulation), diseases of the lungs, etc.

2.11.1.2 White blood cell tests

The other major type of blood cells is the white blood cells (WBC), which are also referred to as leukocytes. White blood cells are bigger than red blood cells but fewer in number. For every leukocyte present in a sample there will normally be 600 to 700 RBC. The major role of the white blood cells is to defend the body against invading organisms such as bacteria, viruses, and fungi. White cell count estimates the total number of white blood cells per litre of blood. An abnormal WBC count may indicate an infection, inflammation, or other stress in the body. For example, a bacterial infection can cause the WBC count to increase, or decrease, dramatically. The number of WBC is typically elevated when the body is fighting a severe infection or stressed by metabolic toxins (a patient that was in acute kidney failure with waste products building up in its body would normally have an elevated WBC). In addition, when extremely excited (if an animal was overly excited or frightened when drawing the blood sample), white blood cells will be released into the blood and the levels will rise. Abnormally high levels of white blood cells may indicate also tissue damage, leukaemia, or inflammatory diseases. The WBC count will be lower than normal, if an animal has been weakened from a prolonged, debilitating disease and in some viral infections. A number of viral infections can cause a temporary reduction in the white cell count. Abnormally low numbers of white blood cells may indicateliver or spleen disorders, bone marrow disorders, or exposure to radiation or toxic substances.

There are different types of leukocytes, and a white blood count (WBC) is a total of all the various kinds. The white blood cell differential assesses the ability of the body to respond to and eliminate infection. It also detects the severity of allergic and drug reactions plus the response to parasitic and other types of infection. It is essential in evaluating the reaction to viral infections and response to chemotherapy. It can also identify various stages of leukemia.

White blood cells are divided into two groups depending on how they react to the stains that are used to better observe them under a microscope. There are granulocytes (they are WBC with granules that absorb the stain) and the agranulocytes (those that do not absorb the stain). The granulocytes include the neutrophils, eosinophils, and the basophils, while the agranulocytes are the lymphocytes and monocytes. Each type of cell plays a different role in protecting the body. The numbers of each one of these types of white blood cells

give important information about the immune system. Too many or too few of the different types of white blood cells can help fight an infection, an allergic or toxic reaction to medicines or chemicals, and many conditions, such as leukemia.

***Neutrophils* or polymorphonuclear cells (Polys or Neuts)**:Neutrophils are the main phagocytic cell of the human immune system (van Raam *et al*., 2008). These cells are the first to arrive at a site of infection to kill ingested microorganisms (van Raam *et al*., 2008). Neutrophils derive most of the energy required for these and other functions from a high rate of glycolysis (Roos *et al*., 2003). This ensures that neutrophils can function in an inflammatory environment where the oxygen tension may be low or even absent (Borregaard and Herlin, 1982; Peyssonaux and Johnson, 2004). Neutrophils are also formed in the bone marrow. They normally account for 55% to 70% of WBCs. Mature cells have a multi-lobed nucleus and are referred to as 'segmented cells' (sometimes called 'segs'), while the immature ones have a single-lobed nucleus and are referred to as 'bands.' The bands are younger than the segs - when first released from the marrow neutrophils are bands, and after spending time in the circulating blood they mature into segs. These cells function by actually engulfing disease-causing bacteria and other small particles. In the presence of a bacterial infection, their number in the peripheral blood increases, the bone marrow releases more of the young cells into the circulation, and the percentage of bands increases in relation to the segmented ones. When this occur, it implies that there is more severe reaction, since the body is releasing more and more immature cells into the circulation to defend itself against the infection. Abnormally high neutrophil percentage could indicate eclampsia, acute infection, rheumatic fever, rheumatoid arthritis, myelocytic leukemia, gout, trauma, thyroiditis or stress. An abnormally low level of neutrophils may signify influenza, chemotherapy, aplastic anemia, radiation therapy or exposure to radiation, or widespread bacterial infection. In most viral infections, the total number of neutrophils decreases.

Eosinophils: Eosinophils are usually seen in fewer numbers than neutrophils. They are normally 1% to 4% of WBCs.They are also produced in the bone marrow. They have the ability to engulf foreign particles into their bodies. *Eosinophils* can increase in response to allergic disorders, inflammation of the skin, and parasitic infections. They can also increase in response to various bone marrow disorders. Decreased levels of eosinophils

can occur as a result of stress, steroid exposure and anything that may suppress WBC production generally.

Basophils:The last of the granulocytes is the basophil. These are the least common of all the WBC's.They are usually less than 1% of WBCs. In many samples, none are present. These cells can digest bacteria and other foreign bodies (phagocytosis). They are produced with the bone marrow and also take part in allergic responsessuch as asthma or skin allergies. Increased basophil production may be associated with bone marrow disorders or viral infection. They can increase in cases of leukemia, chronic inflammation, the presence of a hypersensitivity reaction to food, or radiation therapy. Diminished basophil counts are associated with stress reactions, some allergic reactions, hyperthyroidism, and prolonged steroid exposure.

Lymphocytes:Of the agranulocytes, the most abundant is the lymphocyte. Lymphocytes are normally 20% to 40% of WBCs. These cells play both an immediate and delayed role in response to infection or inflammation. They are formed and released from lymphoid tissue such as lymph nodes, spleen, etc. They engulf organisms, but fulfill their function of defending the body in other ways. The lymphocytes can be divided into two major types by their functions - B cells and T cells. The B cells produce antibodies, which are protein molecules that attach to and thereby destroy invading organisms or other foreign materials and particles. The T cells activate and help other cells destroy viruses and other foreign material. When lymphocytes numbers decrease it is referred to as a lymphopenia, and is frequently noted in the initial stages of infections (a common example would be parvovirus) or following the use of corticosteroids like prednisone. Decreased lymphocyte levels can indicate diseases that affect the immune system (such as renal failure lupus, some cancers, immunodeficiency such as the later stages of HIV infection), exposure to radiation,chemotherapy, or sepsis. There are other situations that bring about reduced lymphocyte numbers, but they are fairly uncommon. An increase in the number of lymphocytes does not happen as consistently as might be expected, but is noted in prolonged illnesses. Examples of this would be when bacterial or viral infections have gone on for a long time or in certain autoimmune diseases. Too many lymphocytes may mean infectious hepatitis, a chronic bacterial infection, multiple myeloma, lymphocytic leukemia, infectious mononucleosis, recovery from a bacterial infection, or a viral

infection. A common cause of increased lymphocytes is leukemia, which is a cancer of blood cell production that is usually fatal.

Monocytes or Macrophages (Monos) make up 2% to 8% of WBC. Monocytes develop and are stored in the spleen and bone marrow. They have the ability to engulf foreign material, such as infectious organisms. Additionally, they secrete various protein molecules that help in the clean up of inflamed and irritated tissue. Monocytes circulate in the blood.Their numbers do not vary greatly unless there is a cancerous leukemia condition affecting their cell lines. When monocytes settle in various tissues they are called macrophages. Monocytelevels can increase in response to infection of all kinds as well as to inflammatory disorders. An abnormally high level of monocytes may mean bacterial infection, viral infection, tuberculosis, parasitic infection or a chronic inflammatory disease. Monocyte counts are also increased in certain malignant disorders, including leukemia. Decreased monocyte levels can indicate bone marrow injury or failure and some forms of leukemia.

Platelets (PT) or thrombocyte*:* These are the smallest type of blood cell. The platelets and a protein called fibrinogen are responsible for the repair of all damaged blood vessels. Platelets play an important role in blood clotting and the prevention of bleeding. When a blood vessel is damaged or cut, platelets clump together and form scabs until the blood clots. If there are too few platelets, uncontrolled bleeding may be a problem. Platelets are almost never so high that they cause health problems. If there are too many platelets, there is a chance of a blood clot forming in a blood vessel. The platelet count is the number of platelets in a given volume of blood. Both increases and decreases can point to abnormal conditions of excess bleeding or clotting. Abnormally low numbers of platelets is known as thrombocytopenia, while an abnormally high level of platelets is known as thrombocytosis. Platelet counts are often used to monitor medications that can have toxic effects on bone marrow, or conditions such as thrombocytopenia. They may also be used to help diagnose problems associated with abnormal bleeding or bruising.

2.11.2 Blood Protein

Serum proteins

The most abundant compounds in the serum are proteins. Amino acids are the building blocks of all proteins. In turn, proteins are the building blocks of all cells and body tissues. They are the basic components of enzymes, many hormones, antibodies and clotting agents. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, proteins help balance the osmotic pressure of the blood and tissue. Osmotic pressure is part of what keeps water inside a particular compartment of your body. Proteins play a major role in maintaining the delicate acid-alkaline balance of the blood. Finally, serum proteins serve as a reserve source of energy for body tissues and muscle when an adequate amount is not ingested.

The major measured serum proteins are divided into two groups, albumin and globulins. There are four major types of globulins, each with specific properties and actions. A typical blood panel will provide four different measurements - the total protein, albumin, globulins, and the albumin-globulin ratio.

***Total Protein*:**Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney disease, liver disease, and many other conditions. Sometimes, conditions are first detected with routine testing before symptoms have begun to appear. If total protein is abnormal, further tests must be performed to identify which specific protein is abnormally low or high so that a specific diagnosis can be made.

A total protein test is one component of a Comprehensive Metabolic Panel (CMP) that is often ordered as part of a routine health checkup. Total protein may also be ordered to provide general information about the nutritional status, such as when a recent weight loss has been undergone, and/or conditions involving major organs, such as the kidney and liver to investigate the cause of abnormal pooling of fluid in tissue (edema). However, if results are abnormal, further testing is usually required to help diagnose the disease affecting protein levels in the blood. Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption, such as Celiac disease or inflammatory bowel disease (IBD). High total protein levels may be seen with chronicinflammation or infections such as viral hepatitis or HIV. They may be caused by bone marrow disorders such as multiple myeloma. Total protein may be elevateddue to chronic infection (including tuberculosis), adrenal cortical

hypofunction, liver dysfunction, collagen vascular disease (rheumatoid arthritis, systemic lupus, scleroderma), hypersensitivity states, sarcoidosis, dehydration (diabetic acidosis, chronic diarrhea, etc.), respiratory distress, hemolysis, cryoglobulinemia, alcoholism, and leukemia. Total protein may be decreased due to malnutrition and malabsorption (insufficient intake and/or digestion of proteins), liver disease (insufficient production of proteins), diarrhea (loss of protein through the GI tract), severe burns (loss of protein through the skin), hormone Imbalances that favor breakdown of tissue, loss through the urine in severe kidney disease (proteinuria), low albumin, low globulins, and pregnancy (dilution of protein due to extra fluid held in the vascular system).

Because the total protein represents the sum of albumin and globulins, it is more important to know which protein fraction is high or low than what is the total protein.

Albumin: Albumin is synthesized by the liver using dietary protein. It is the main constituent of total protein; the remaining fraction is called globulin (including the immunoglobulins).Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. It is a very strong predictor of health; low albumin is a sign of poor health and a predictor of a bad outcome.
Albumin levels may be elevated in dehydration – actual, congestive heart failure, poor protein utilization, and glucocorticoid excess (can result from taking medications with cortisone effect, the adrenal gland overproducing cortisol, or a tumor that produces extra cortisol like compounds). Albumin levels may be decreased in dehydration, hypothyroidism, chronic debilitating diseases (ex: RA), malnutrition - Protein deficiency, dilution by excess water (drinking too much water, which is termed “polydipsia,” or excess administration of IV fluids), kidney losses (Nephrotic Syndrome), protein losing-enteropathy (protein is lost from the gastrointestinal tract during diarrhea), skin losses (burns, exfoliative dermatitis), liver dysfunction (the body is not synthesizing enough albumin and indicates very poor liver function), insufficient anabolic hormones such as Growth Hormone, DHEA, testosterone, etc.

Globulins, Total serum: Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. The specific profile of the globulins is determined by protein electrophoresis (SPEP), which separates the proteins according to size and charge. There are four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins, and alpha-1 globulins. Once the abnormal

group has been identified, further studies can determine the specific protein excess or deficit. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low. Antibodies are produced by mature B lymphocytes called plasma cells, while most of the other proteins in the alpha and beta fractions are made in the liver.

The globulin level may be elevated in chronic infections (parasites, some cases of viral and bacterial infection), liver disease (biliary cirrhosis, obstructive jaundice), carcinoid syndrome, rheumatoid arthritis, ulcerative colitis, multiple myelomas, leukemias, Waldenstrom's macroglobulinaemia, autoimmunity (Systemic lupus), collective-tissue diseases, and kidney dysfunction (Nephrosis). The serum globulin level may be decreased in Nephrosis (A condition in which the kidney does not filter the protein from the blood and it leaks into the urine), alpha-1 antitrypsin deficiency (Emphysema), acute hemolytic anemia, liver dysfunction, hypogammaglobulinaemia/Agammaglobulinaemia.

A/G (albumin/globulin) ratio:The liver can function adequately on 20% of liver tissue, thus early diagnosis by lab methods is difficult. A reversed A/G Ratio may be a helpful indicator. With severe liver cell damage, the prolonged prothrombin time will not change with ingestion of Vitamin K. The proper albumin to globulin ratio is 2:1. Because disease states affect the relative changes in albumin and globulins in different ways, this may provide a clue to the cause of the change in protein levels. When <1.7, there is may be a need for increasing stomach acidity. When >3.5 there may be a need for stomach acidity and pepsin.Optimal range is 1.7-2.2. The AG ratio may be elevated in hypothyroidism, high protein/high carbohydrate diet with poor nitrogen retention, hypogammaglobulinemia (low globulin), glucocorticoid excess (can be from taking medications with cortisone effect, the adrenal gland overproducing cortisol, or a tumor that produces extra cortisol like compounds, low globulin). The AG ratio may be decreased in liver dysfunction.A low A/G ratio may reflect overproduction of globulins, such as seen in multiple myeloma or autoimmune diseases, or underproduction of albumin, such as occurs with cirrhosis, or selective loss of albumin from the circulation, as occurs with kidney disease (nephrotic syndrome). A high A/G ratio suggests underproduction of immunoglobulins as may be seen in some genetic deficiencies and in some leukemia. More specific tests, such as albumin, liver enzyme tests, and serum protein electrophoresis must be performed to make an accurate diagnosis.

Liver function tests (LFTs or LFs), which include liver enzymes, are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. Liver function tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment.

Aspartate transaminase (AST): This is also called Serum Glutamic Oxaloacetic Transaminase (SGOT) or aspartate aminotransferase (ASAT). The blood test for aspartate aminotransferase (AST) is usually used to detect liver damage. It is raised in acute liver damage, but is also present in red blood cells, cardiac and skeletal muscle, and is therefore not specific to the liver. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. Low levels of AST are normally found in the blood. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days. Some herbs and natural products (such as Echinacea and valerian) can affect AST results. Sometimes AST may be used to monitor people who are taking medications that are potentially toxic to the liver. If AST levels increase, then the person may be switched to another medication. It is often ordered in conjunction with another liver enzyme, alanine aminotransferase (ALT), or as part of a liver panel to screen for and/or help diagnose liver disorders. AST and ALT are considered to be two of the most important tests to detect liver injury, although ALT is more specific than AST. Sometimes AST is compared directly to ALT and an AST/ALT ratio is calculated. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage (Nyblom *et al*., 2004; Nyblom *et al*., 2006).

Alanine transaminase (ALT), also called Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. ALT rises dramatically in acute liver damage, such as viral hepatitis or paracetamol (acetaminophen) overdose. Elevations are often measured in multiples of the upper limit

of normal (ULN). In most types of liver disease, the ALT level is higher than AST, and the AST/ALT ratio will be low. There are a few exceptions. The AST/ALT ratio is usually increased in alcoholic hepatitis, cirrhosis, and in the first day or two of acute hepatitis or injury from bile duct obstruction.

Blood Urea Nitrogen (BUN) is nitrogen in the form of urea in the blood or serum, used as an indicator of kidney function. It is a byproduct from the breakdown of blood, muscle and protein that is formed in the liver and collects in the bloodstream; patients with kidney failure have high BUN levels.Urea forms in the liver as the end product of protein metabolism, circulates in the blood, and is excreted through the kidney in urine. The BUN, determined by a blood test, is directly related to the metabolic function of the liver and the excretory function of the kidney. It is a measure of kidney function and state of hydration. BUN may be lower than normal in over hydration, liver failure, acromegally, pregnancy and low protein diet. It is more of concern if it is elevated. Increased BUN often indicates early kidney damage, as well as dehydration, gastrointestinal bleeding, starvation, shock or urinary tract obstruction–by tumor or prostate gland; Decreased BUN may indicate liver disease, malnutrition or a low protein diet.

Thecreatinine blood test is used along with a BUN (blood urea nitrogen) test to assess kidney function. Both are frequently ordered as part of a basic or comprehensive metabolic panel (BMP or CMP), groups of tests that are performed to evaluate the function of the body's major organs. BMP or CMP tests are used to screen healthy people during routine physical exams and to help evaluate acutely or chronically ill patients in the emergency room and/or hospital. If the creatinine and BUN tests are found to be abnormal or if there is the presence of an underlying disease, such as diabetes, that is known to affect the kidneys, then these two tests may be used to monitor the progress of kidney dysfunction and the effectiveness of treatment. Blood creatinine and BUN tests may also be ordered to evaluate kidney function prior to some procedures, such as a CT (computed tomography) scan, that may require the use of drugs that can damage the kidneys.

Increased creatinine levels in the blood suggest diseases or conditions that affect kidney function, such as damage to or swelling of blood vessels in the kidneys (glomerulonephritis) caused by, for example, infection or autoimmune diseases, bacterial infection of the kidneys (pyelonephritis), death of cells in the kidneys' small tubes (acute

tubular necrosis) caused, for example, by drugs or toxins, prostate disease, kidney stone, or other causes of urinary tract obstruction, and reduced blood flow to the kidney due to shock, dehydration, congestive heart failure, atherosclerosis, or complications of diabetes. Creatinine blood levels can also increase temporarily as a result of muscle injury and are generally slightly lower during pregnancy. Low blood levels of creatinine are not common, but they are also not usually a cause for concern. They can be seen with conditions that result in decreased muscle mass.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 PURCHASE OF VEGETABLES

The vegetables which were purchased from Nwaiwu family gardenin Ijora, Lagos, werecultivated without fertilizer. They were later identified and authenticated by a botanist at Botany Department, University of Ibadan as:

1. *Gnetum africanum*
2. *Ocimum gratissimum*

3.2PROCEDURES FOR AQUEOUS EXTRACTION OF THE TWO VEGETABLES (*GNETUM AFRICANUM AND OCIMUM GRATISSIMUM*)

PROCEDURE

The fresh vegetable leaves were picked, washed with distilled water and ground into paste using pestle and mortar after which, the paste was poured in a clean cloth mesh and squeezed thoroughly to obtain the extract. The volume of the extract was taken using a measuring cylinder. This was done for the two vegetables. Gloves were worn through out the procedure to reduce contamination. The extracts were then taken to the International Institute of Tropical Agriculture (IITA) for lyophilizing.

3.3 LYOPHILIZATION (FREEZE DRYING) OF VEGETABLES

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

3.4 PREPARATION OF THE STOCK AND WORKING CONCENTRATION:

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

3 mg of each vegetable extract was also dissolved in 100mls of distilled water and kept in a refrigerator.

WORKING CONCENTRATION:

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

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

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

3.5 EXPERIMENTAL ANIMALS

Thirty (30) adult albino rats were distributed randomly into 6 groups. Five (5) experimental groups and One (1) control group.

The animals, which were from the same litter, were purchased from the animal house, Physiology Department, University of Ibadan. They were transferred to the Animal House inInstitutes for Medical Research and Training(IMRAT), 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 until a range of between 160g and 280g was obtained. They were kept in iron cages whose dimensions are 30 cm by 15 cm by 25cm at room temperature. The cages and water bottles were washed thoroughly while the sawdust which served as their beddings was treated with dettol and sun dried, and changed periodically. The rats were subsequently distributed randomly and placed in one control and five experimental groups.

Group 1: - this was the control group and they were maintained without any of theextracts or cyanide.

Group 2: - this group was fed with 3 mg/kg body weightof cyanide.

Group 3: - this group was fed with equal (3 mg/kg body weight)weight cyanide and lyophilized*Gnetum africanum* extract each.

Group 4: - this group was fed with an equal (3 mg/kg body weight)weight cyanide and lyophilized *Ocimum gratissimum* extract each.

Group 5: - this group was fed with3 mg/kg body weightof lyophilized*Gnetum africanum* extract only.

Group 6: - this group was fed with 3 mg/kg body weightof lyophilized*Ocimum gratissimum* extract only.

All the rats in each group were fed adlibitum with commercial rat pellet and water daily.

3.5.1 IDENTIFICATION OPERATION STRATEGY

Each rat across the groups were marked at notable parts of the body, using an indelible marker for ease of dentification, as follows:

1. Head
2. Neck
3. Trunk
4. Base
5. Tail

The animals were picked from the tail and the flesh of the back neck is gripped with the left hand and turned upwards with its limbs hanging up and the tail tucked between the hollow of the left hand, then a clear passage to the throat was sought before the drugwas administered. Before drug administration, physical (i.e. average body weight change, feed intake and water intake) and physiological parameters (i.e. agility, fur colour, nasal discharge and ocular lesion) were observed and noted. The procedure was followed for 14 days.

3.5.2 OBSERVATIONS

Observation of the rats in all the groups was made each day to record changes in

1. Amount of water consumption
2. Amount in feed intake
3. Body weight
4. Physical characteristics

Plate 3.2:Researcher administering drugswithoral canular to the rats

3.6EXPERIMENTAL PROCEDURE

3.6.1 PROCEDURE FOR ADMINISTERING KCN AND THE VEGETABLE EXTRACTS

The animals were fed by gavage 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 daily along with other physical observations before the KCN and the vegetable extracts were administered. Volume of the KCN and the extracts administered was based on the weight of the rats.The labelling was as follows:

Group1:Control

Group 2: CN only

Group 3: CN+GA

Group 4: CN+OG

Group 5: GA only

Group 6: OG only

Where

Control: distilled water only

CN: cyanide

GA: *Gnetum africanum* extract

OG: *Ocimum gratissimum* extract

Plate 3.3:Blood collected through ocular puncture for biochemical and haematological analysis

3.6.2 COLLECTION OF BLOOD SAMPLES

The blood samples were collected after 14 days of experimental period.The rats were fasted for 24 hours before the blood samples were collected. Capillary tubes were used to collect blood samples from the rats while they were still alive using the ocular punctures method. The blood samples were placed inside Lithium heparinized bottles and were centrifuged at 1000 revolution/ minutes on table centrifuge at room temperature, after which the serum was picked and decanted into universal bottles which were taken to the Chemical Pathotology Department in the University of Ibadan, Ibadan, Oyo State, forHaematologicaland Biochemical analysis.

3.6.2.1 Determination of Heamatological Parameters

Haematological examination was done using the International Council forStandardisation inHaematology(ICSH) standard procedures.

3.6.2.2 Determination of Biochemical Parameters

Total protein was determined by the Lowry method (1951).Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined using Randox test kits (Reitman and Frankel, 1957).

.3.7 DATA AND STATISTICAL ANALYSIS

Thedaily record of body weight, feed andwater consumption, haematological and biochemical datawere subjected to statistical analysis. The results were analysed using descriptive statistics and Kruskal-Wallis. The results were expressed as mean and standard deviation. In comparing the results of the groups, analysis of variance (ANOVA) and student‘t’ test was applied and the difference was taken to be significant when P-value is <0.05 to ascertain the significance of the intervention.

CHAPTER FOUR

4.0 RESULTS

This chapter presents the outcome of the various treatment exposure and their effects on feed and water consumption, body weight, counts of various haematological parameters, concentration of various liver and kidney function indicators post-experiment, and the physical health of the rats. These are presented in tables, figures and plates.

4.1 Effects of treatment exposure on average body weight, feed and water consumption

All rats survived to the end of the study. *Gnetum africanum*-treated rat groups (groups 3 and 5), *Ocimum gratissimum*-treated rat groups (groups 4 and 6) and control group had similar water consumption throughout the experiment. Rats on cyanide treatment onlysignificantly had reduced water consumption. *Gnetum africanum*only (group 5) and control groups had similar feed intake throughout the experiment. On the contrary, groups of rats on cyanide treatment (groups 2, 3 and 4)and *Ocimum gratissimum* only (group 6) halved their feedconsumption.Table 4.1 below presents the average water intake (ml), average feed intake (g) and average body weight (g) of the rats allocated in each group. This indicates the difference between treatment groups in weight gain, feed intake and water consumption.

Table 4.1: Water intake (ml); feed intake (g) and body weight (g) (N=14)

Treatment group	Water intake	Feed intake	Body weight
Control	131.4±41.0[a]	111.0±27.3[a]	88.9±17.9[a]
CN only	91.1±65.7[b]	52.1±16.2[b]	89.4±9.4[a]
CN+GA	137.5±44.5[a]	58.6 ±13.2[b]	95.5±17.3[b]
CN+OG	129.6±28.0[a]	56.7 ±21.5[b]	83.93±13.6[c]
GA only	137.1±25.8[a]	106.9±25.1[a]	98.90±11.7[b]
OGonly	158.6+30.5 [c]	58.7+13.2 [b]	87.21+11.4[ac]

- Values along each column having different superscript letter (s) were significantly different ($p < 0.05$). Values are mean±SD

*CN - Cyanide only

*CN+GA – cyanide and*Gnetum africanum* extract only

*CN+OG – cyanide and*Ocimum gratissimum* extract only

*GA – *Gnetum africanum* extract only

*OG – *Ocimum gratissimum* extract only

4.2. Trend in weight changes between each treatment group and the control within the last five days of drug administration

Figures4.1, 4.3, 4.2,4.4and 4.5 show the trend in weight changes between each treatment group and the control within the last five days of treatment. Group treated with cyanide only had inconsistent weight gain during the initial stage of treatment exposure, but subsequently declined with most rapid weight loss in the last five days of the experiments (Figure 4.1). Cyanide and *Ocimum gratissimum* group had lowered weight gain than the control, but their weight curves turned and subsequently followed a downhill course (Figure 4.3). *Gnetum africanum* treated rat groups gained weight (Figure 4.2 and 4.4) throughout the experiment. Rats maintained on *Ocimum gratissimum* extract only, for extended periods, exhibited a decreasing rate of weight loss and finally adapted to the treatment and maintained constant body weight increase (Figure 4.5).

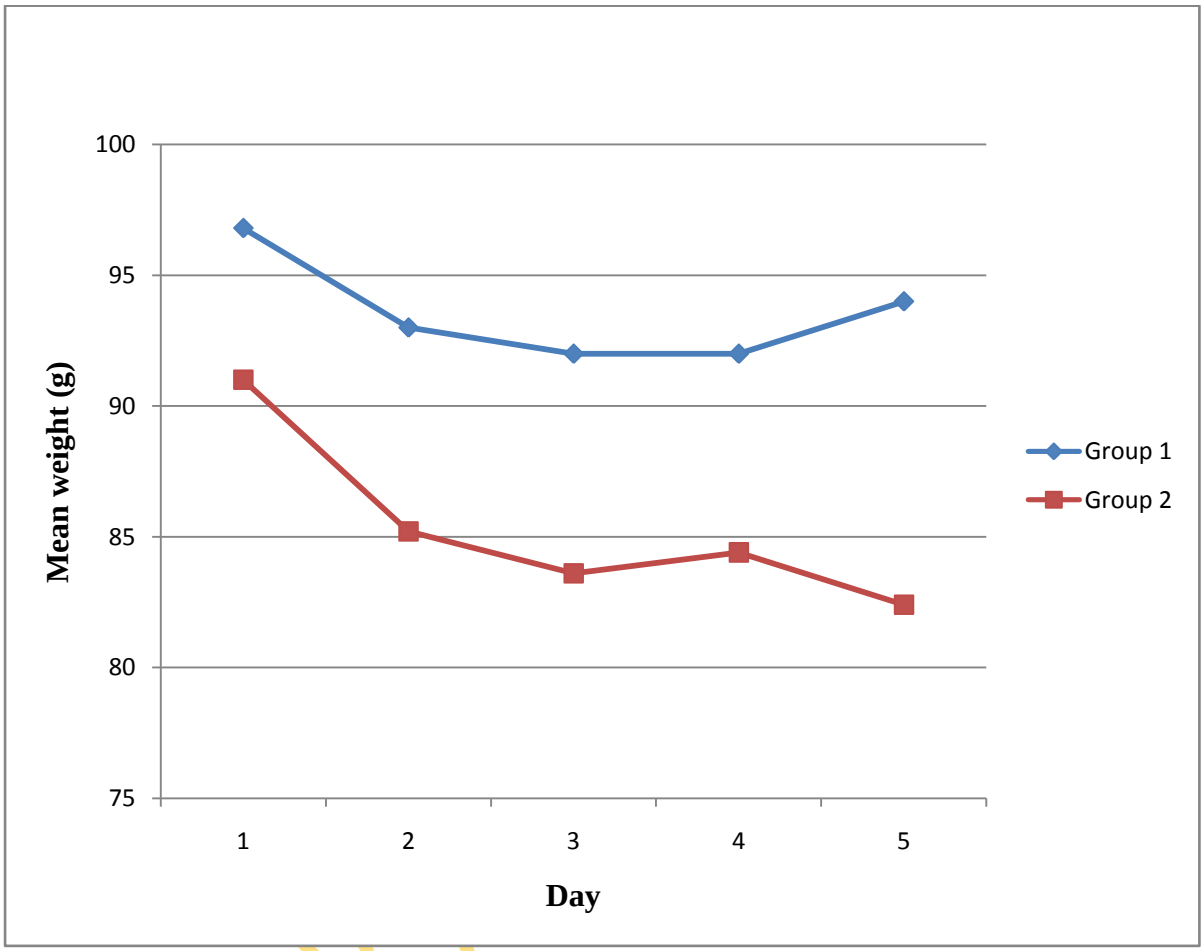


Figure 4.1: Trend in weight change between group 1 and 2 within the last five days of treatment.

Key :

Group1:Control

Group 2: Cyanide only

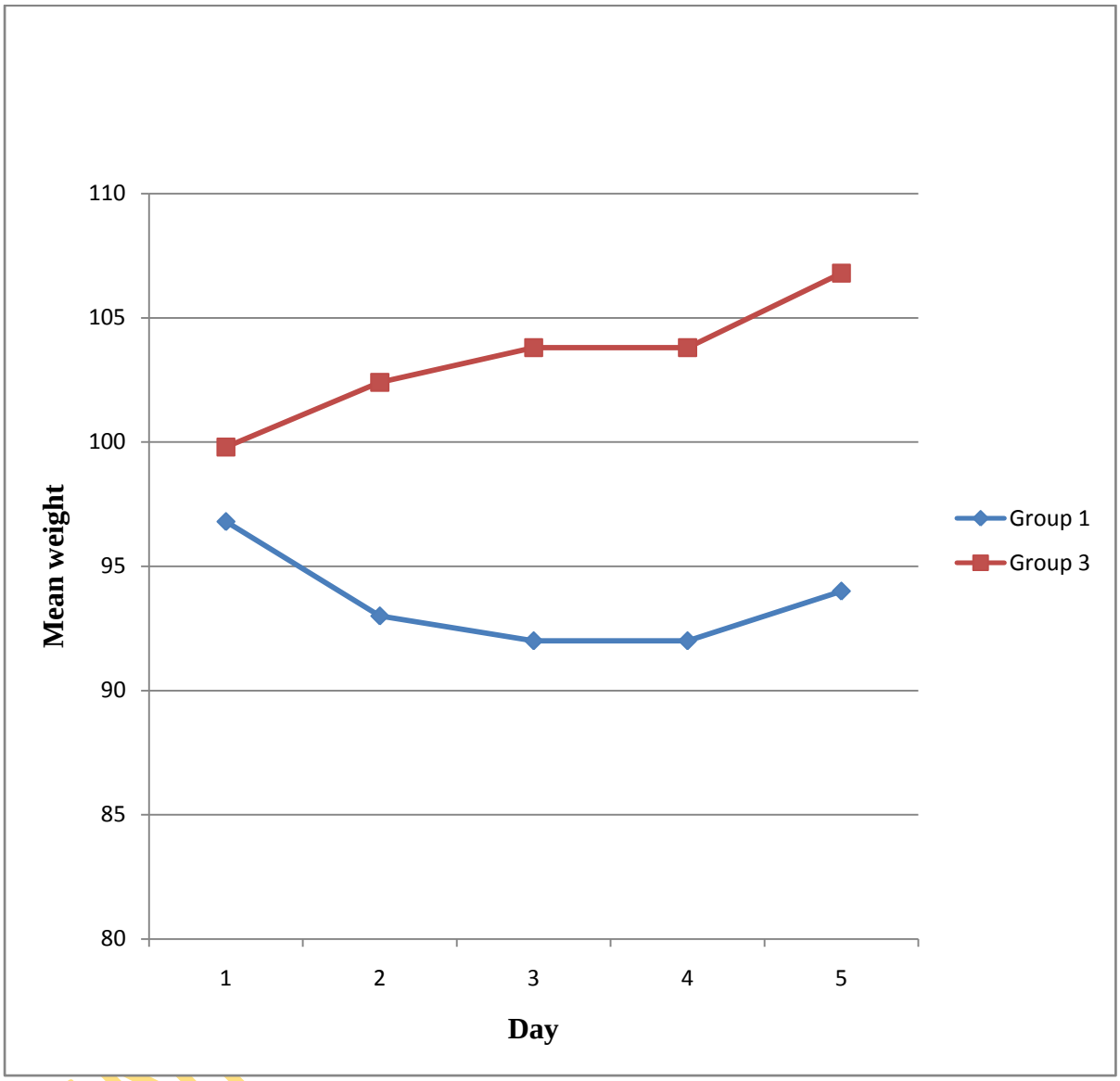


Figure 4.2: Trend in weight change between group 1 and 3 within the last five days of treatment.

Key:

Group1:Control

Group 3: Cyanide and *Gnetum africanum* extract

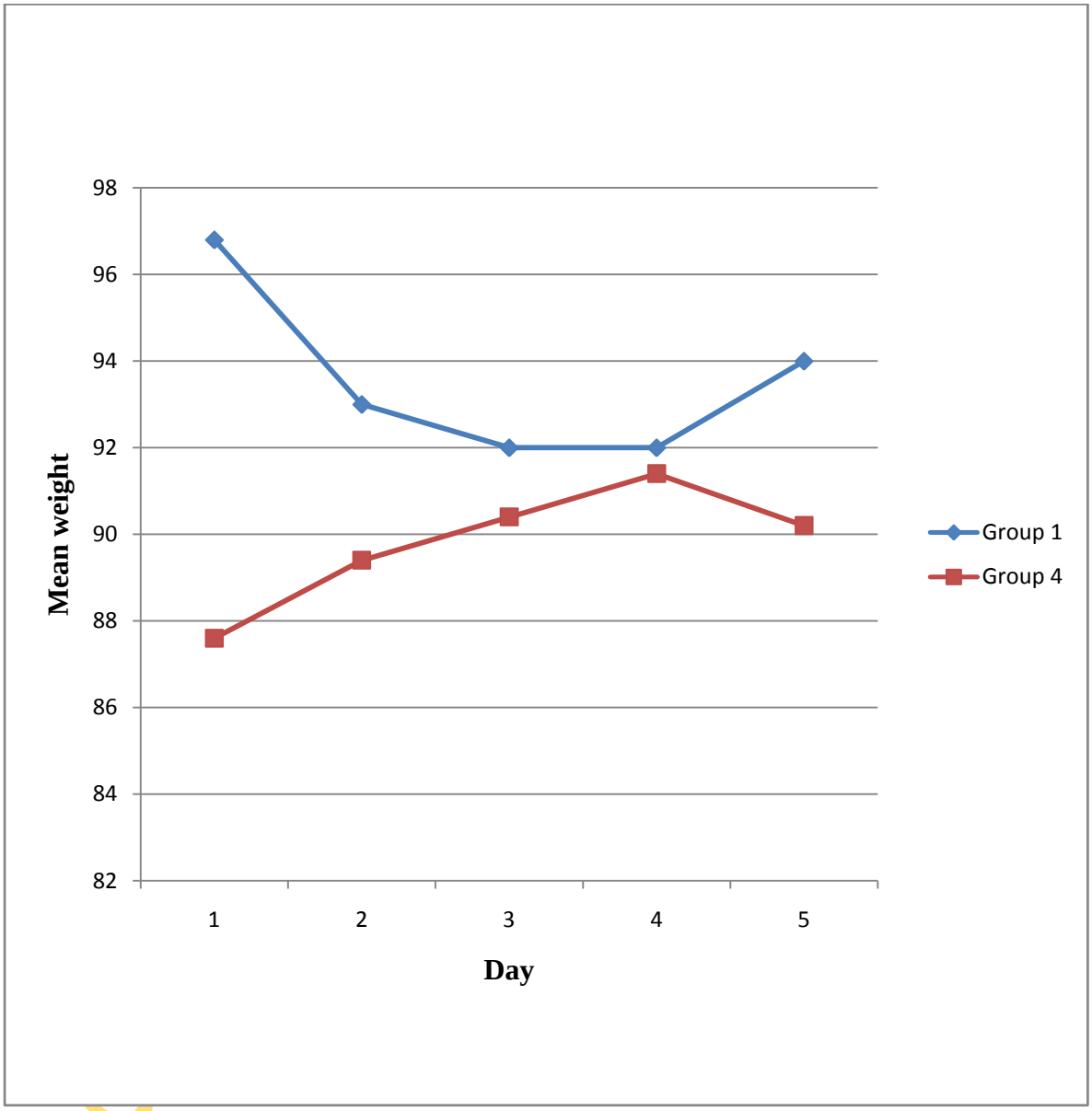


Figure 4.3: Trend in weight change between group 1 and 4 within the last five days of treatment.

Key:

Group1:Control

Group 4: Cyanide and *Ocimum gratissimum* extract

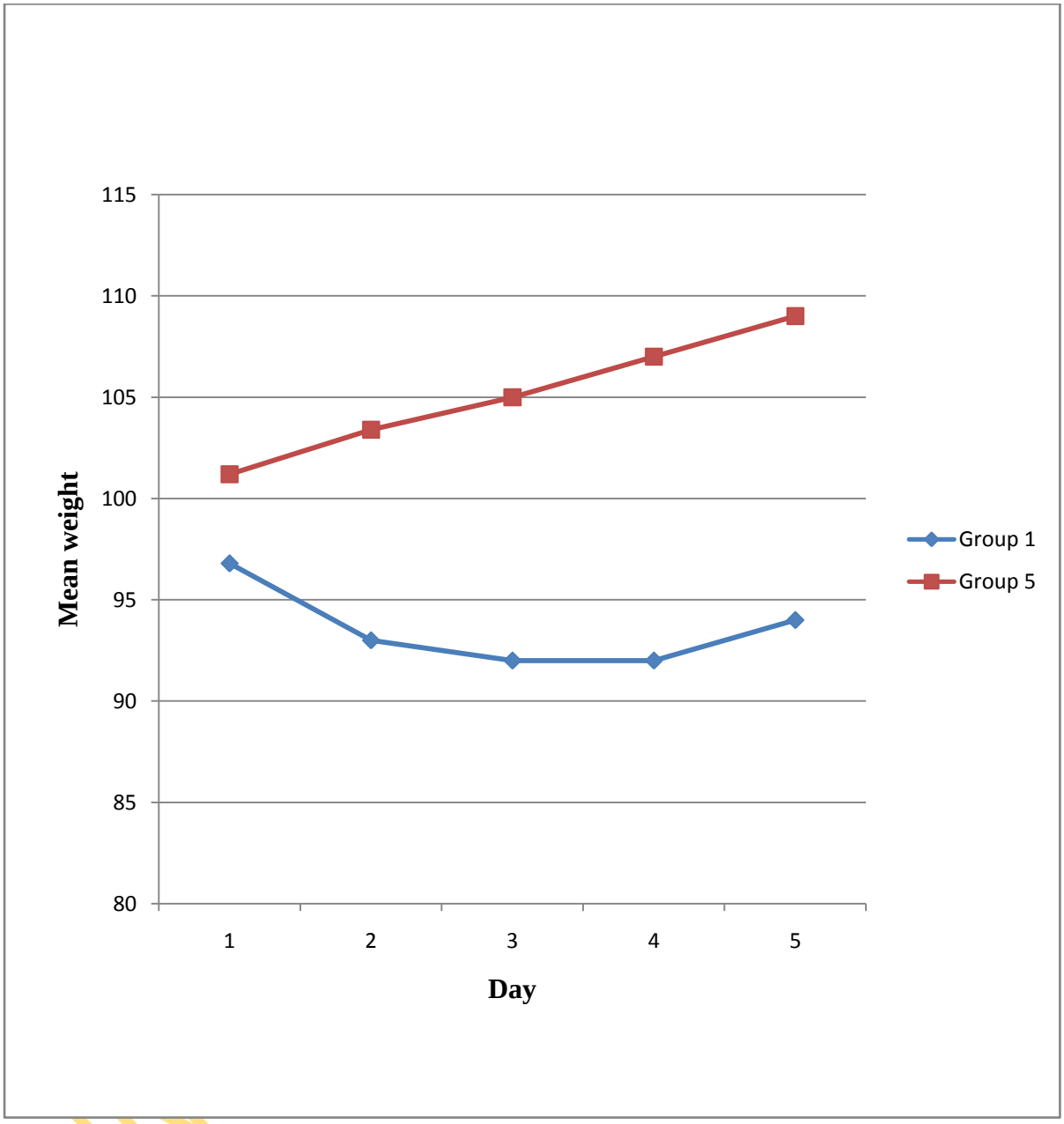


Figure 4.4: Trend in weight change between group 1 and 5 within the last five days of treatment.

Key:

Group1:Control

Group 5:*Gnetum africanum* extract only

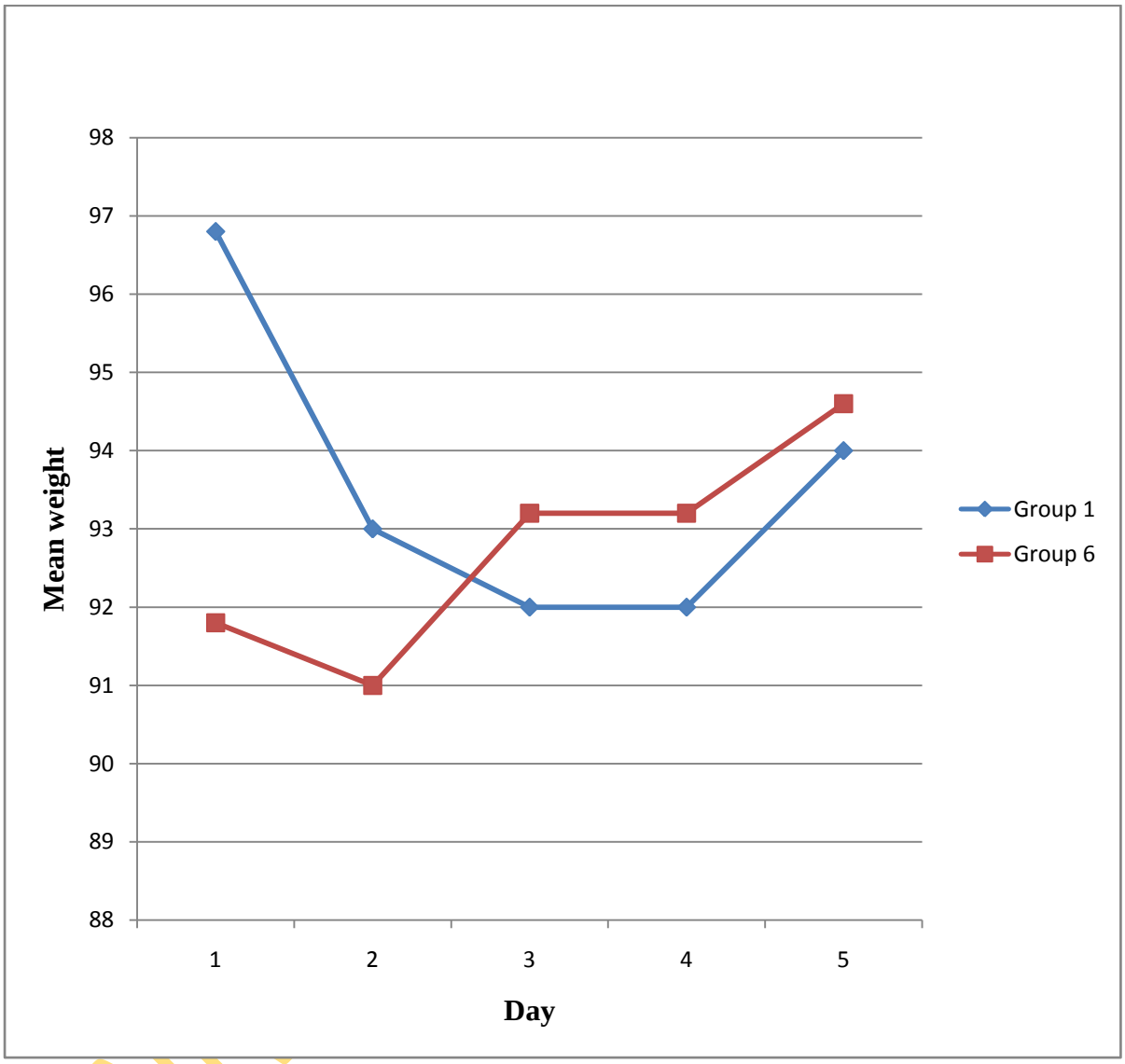


Figure 4.5: Trend in weight change between group 1 and 6 within the last five days of treatment.

Key:

Group1:Control

Group 6:*Ocimum gratissimum* extract only

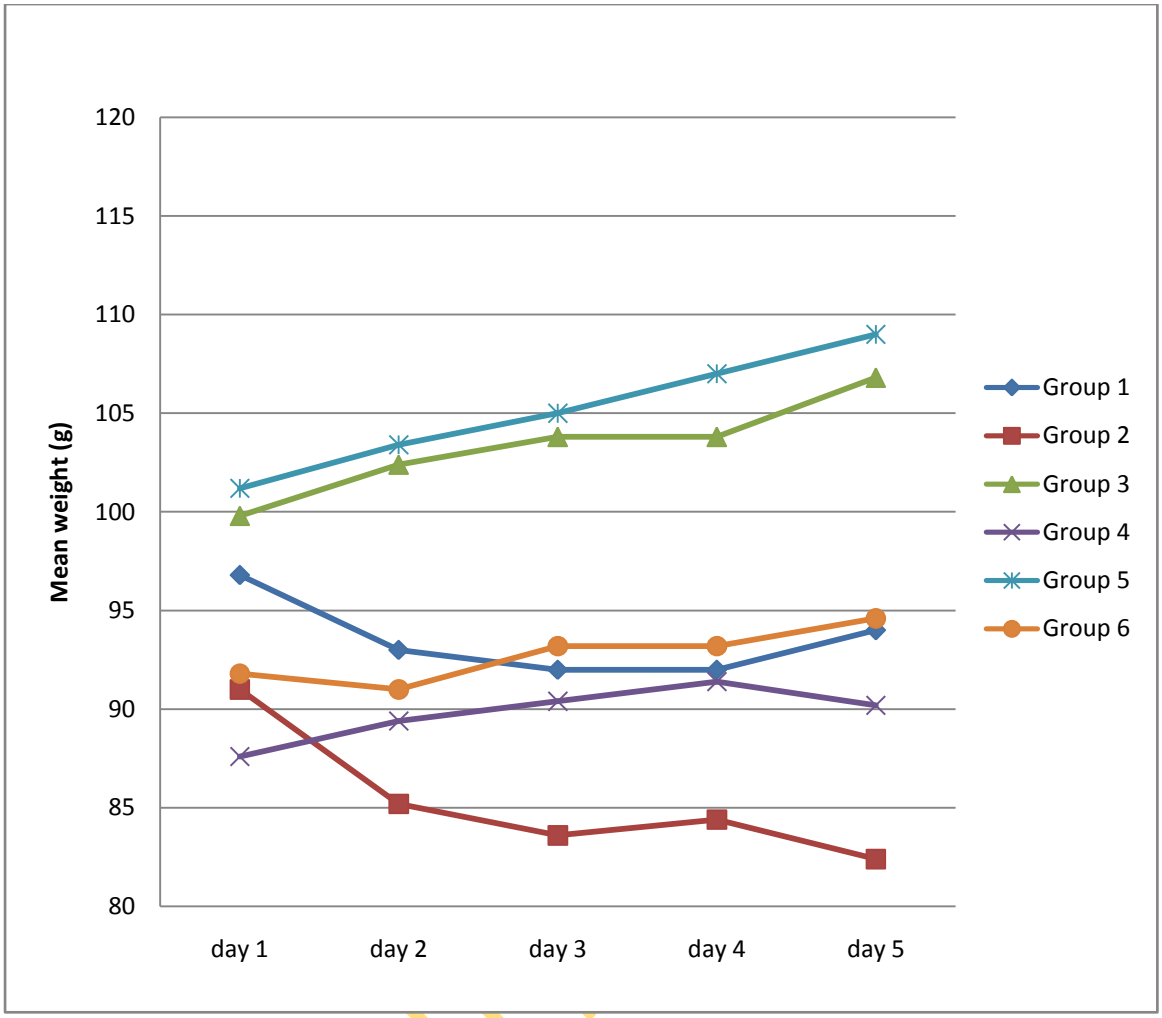


Figure 4.6: Trend in weight change noted in groups 1 to 6 within the last five days of treatment.

Key:

Group1:Control

Group 2: CN only

Group 3: CN+GA

Group 4: CN+OG

Group 5: GA only

Group 6: OG only

Where

Control: distilled water only

CN: cyanide

GA: *Gnetum africanum* extract

OG: *Ocimum gratissimum* extract

4.3. Physical changes during experimental period

Gnetum africanum-treated rat groups thrived and appeared very healthy while they were still gainingweight throughout the experimental period. *Ocimum gratissimum*-treated group of rats appeared healthy but not as *Gnetum africanum*-treated rats. Cyanide-treated rat group appeared less wellwith pale fur, lethargic, and hypoactive. Signs of toxicity were generated and noted in some rats in groups 2 and 4 (plate 4.1 and 4.2).

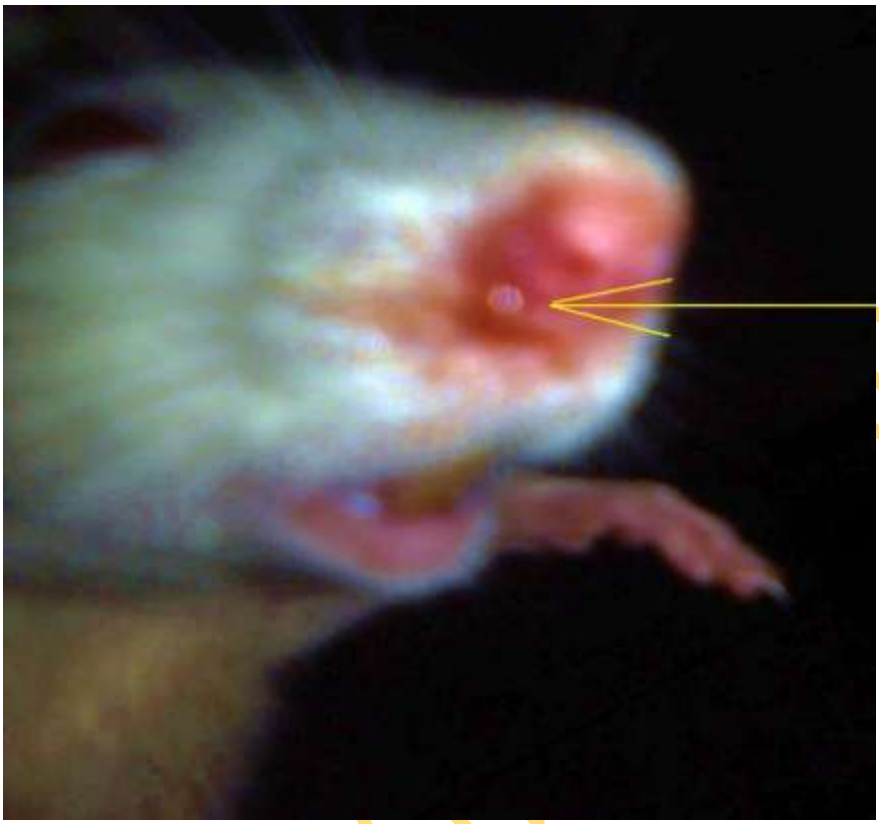


Plate 4.1: An example of rat with nasal discharge/lesion after 14-day treatment with cyanide

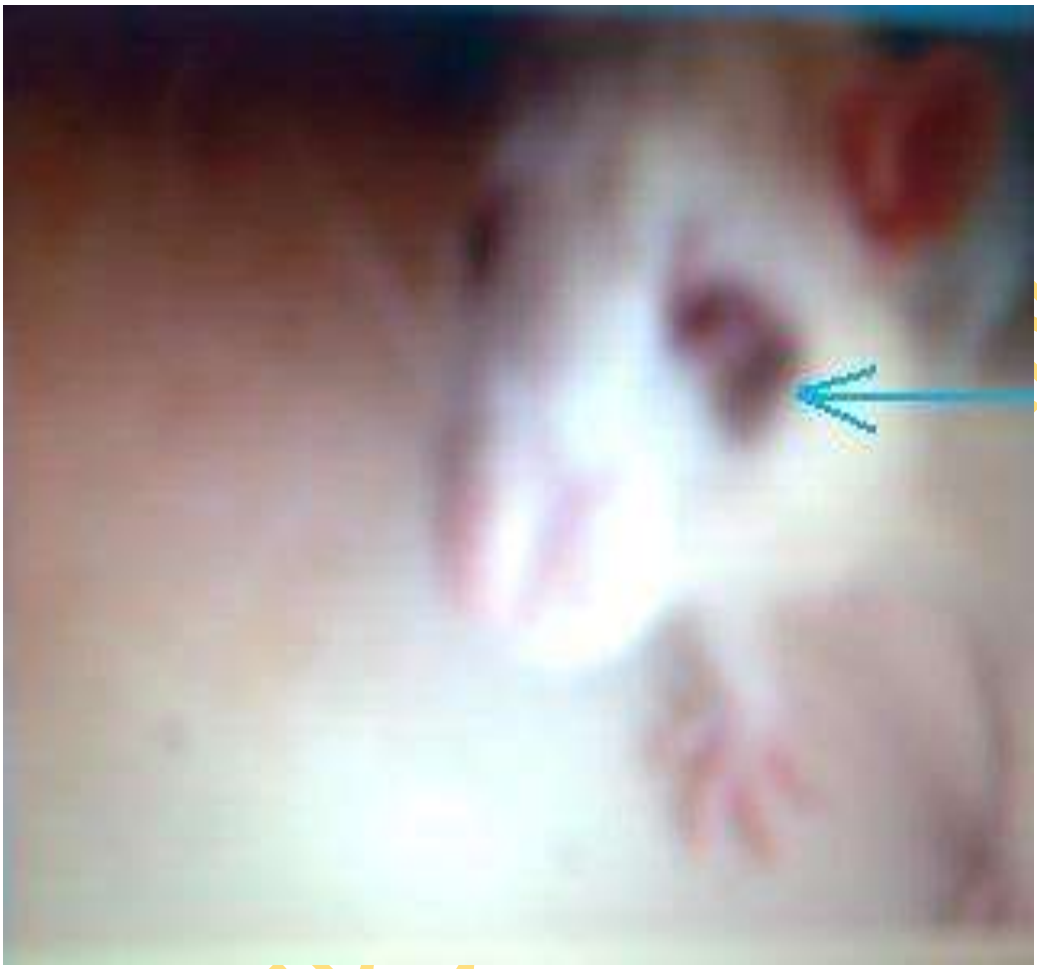


Plate 4.2: An example of rat in group 2 with ocular lesion after 14-day treatment with cyanide

4.4 Predominant signs of cyanide poisoning in treatment groups

Figure 4.6 presents the frequency of nasal discharge in CN only group and CN+OG group. Slimy nasal discharge/lesion was found in 18.6% of rats in group 2 and 10% of rats in group 4. No nasal discharge was found in control, group 3, group 5 and group 6 { n=70 (number of observation)($p<0.05$)}.

Figure 4.7presents the frequency of ocular lesion in cyanide only group.In groups one, three, four, five and six, there was no sign of ocular lesion while in group two, 17.1% of the rats had ocular lesion{n=70 (number of observation)($p<0.05$)}.

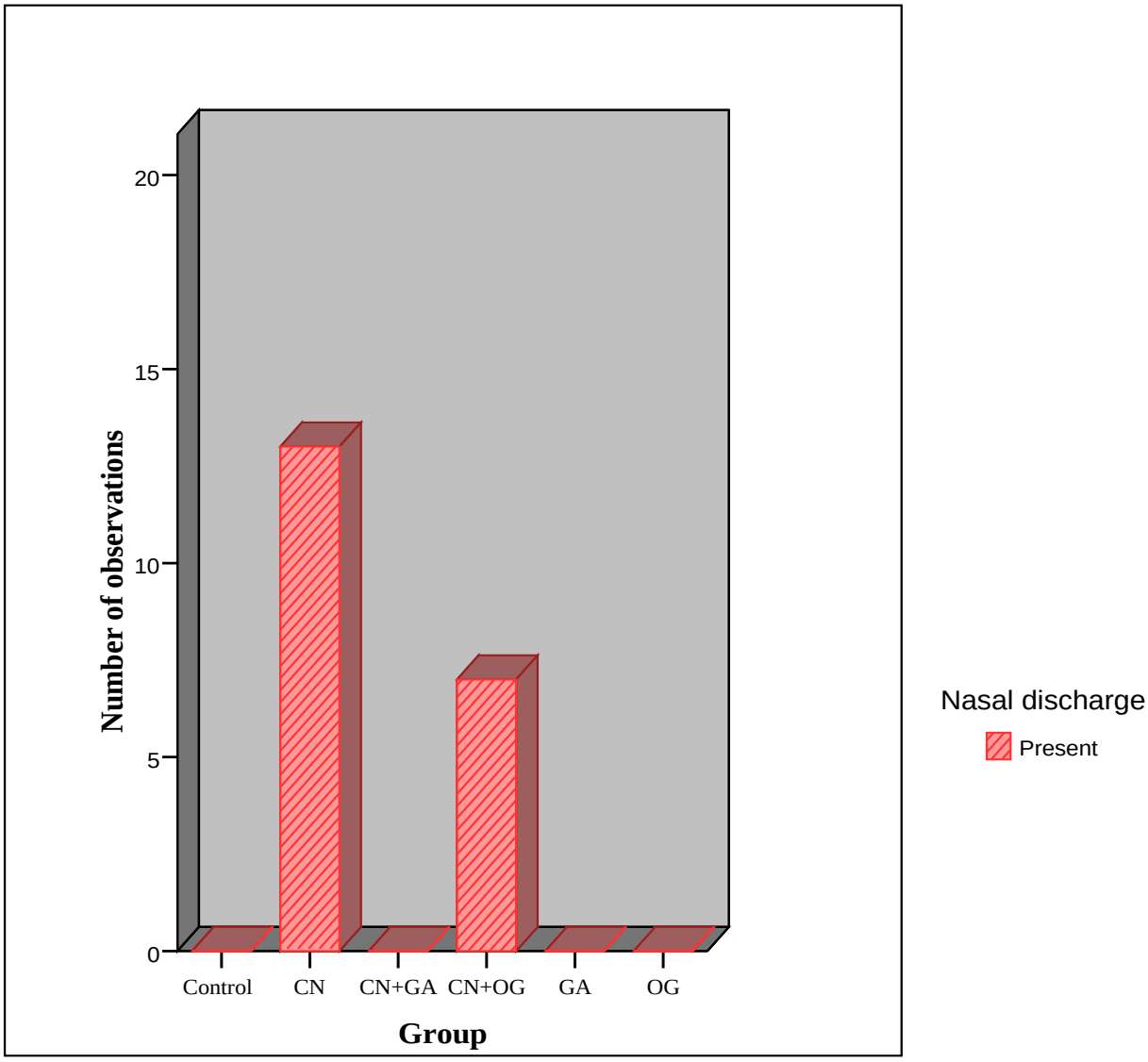


Figure 4.7: Frequency of nasal discharge after treatment

*CN - Cyanide only

*CN+GA – cyanide and*Gnetum africanum* extract only

*CN+OG – cyanide and*Ocimum gratissimum* extract only

*GA – *Gnetum africanum* extract only

*OG – *Ocimum gratissimum* extract only

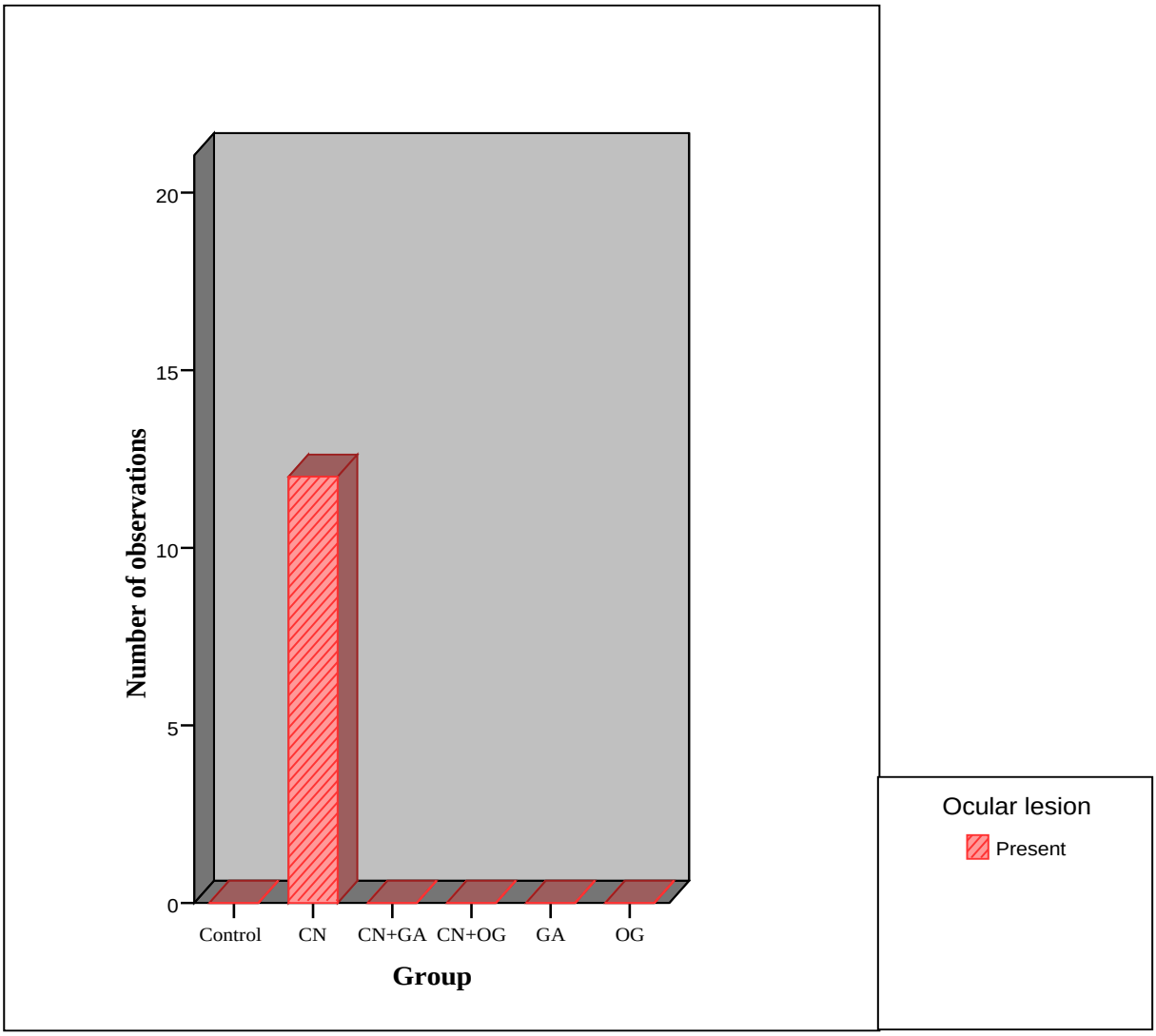


Figure 4.8: Frequency of ocular lesion after treatment

*CN - Cyanide only

*CN+GA – cyanide and*Gnetum africanum* extract only

*CN+OG – cyanide and*Ocimum gratissimum* extract only

*GA – *Gnetum africanum* extract only

*OG – *Ocimum gratissimum* extract only

4.5 Effects of various treatments on haematological and biochemical indicators

Table 4.2 shows the result of the haematological examination of the rats. This reveals the effects of the treatment groups on haematological parameters of the ratsin comparison with the control.

Table 4.3 shows the result of the liver function test of the rats. This reveals the effects of the treatment groups on total protein, globulin, albumin and the levels of liver enzymes detected in the ratswith regards to the control.

Table 4.4 presents the result of the kidney function test of the rats. This reveals the level of blood urea nitrogen and creatinine concentration in serum of treated rats when compared with the control.

Table 4.2: Haematological parameters in blood of rats after 14-day treatment

Treatment group	PCV (%)	Hb (g/dl)	RBC (millions/µlmm^3)	WBC (x10^3) (cell/µlmm^3)	Platelet (x10^4) (cells/ml)	Lym (cell/mm^3)	Neut (cell/mm^3)	Mono (cell/mm^3)	Eosi (cell/mm^3)
Control	45.20±1.48^a	14.46±0.79^a	7.40±0.62^a	5.95±1.55^a	11.06±4.62^a	72.40±4.51^a	21.60±6.15^a	4.40±3.98^a	1.60±1.34^a
CN only	41.20±1.79^b	12.96±0.73^b	6.25±0.5^{bc}	7.70±0.69^a	12.24±1.06^a	70.00±17.38^a	28.00±17.56^a	1.20±0.45^b	0.80±0.84^a
CN+GA	41.20±1.30^b	13.02±0.46^b	6.42±0.66^{ac}	7.21±1.63^a	10.86±2.93^a	66.80±8.08^a	30.80±8.35^a	1.40±0.55^b	1.20±1.64^a
CN+OG	40.80±2.59^b	13.16±0.54^b	5.95±0.60^{bc}	6.67±2.23^a	12.98±5.01^a	73.00 ±10.70^a	23.60±9.18^a	2.20±1.92^{ab}	1.20±1.30^a
GA only	40.80±2.59^b	12.68±0.85^b	6.42±0.36^{ac}	7.63±1.74^a	13.84±3.98^a	67.60±4.10^a	28.60±4.34^a	1.80±0.84^b	2.00±0.71^a
OG only	42.60+4.98^{ab}	14.46+1.59^b	6.88+1.36^{ac}	6.19+2.29^a	9.86+3.74^a	78.20+5.31^a	19.80+5.22^a	1.60+0.89^b	0.80+0.45^a

- Values along each column having different superscript letter (s) were significantly different ($p < 0.05$).
Values are mean±SD

*CN - Cyanide only
*CN+GA – cyanide and*Gnetum africanum* extract only
*CN+OG – cyanide and*Ocimumgratissimum*extract only
*GA – *Gnetum africanum* extract only
*OG –*Ocimumgratissimum*extract only

Table 4.3: Liver function test result of the rats after 14-day treatment

Treatment group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	AST (unit/l)	ALT (unit/l)
Control	5.97±0.15^{a}	2.33±0.71^{ab}	3.63±0.57^{a}	1.67±0.75^{ab}	21.67±5.51^{b}	19.33±4.16^{ab}
CN+DW	4.30±0.44^{b}	3.17±0.25^{a}	1.13±0.25^{b}	0.30±0.10^{a}	19.00±5.29^{b}	21.00±5.29^{b}
CN+GA	4.57±0.15^{bc}	3.23±0.15^{a}	1.07±0.50^{b}	0.30±0.17^{a}	9.67±2.52^{a}	19.00±2.00^{ab}
CN+OG	4.90±0.61^{cd}	3.00±0.30^{a}	1.77±0.25^{b}	0.60±0.00^{a}	14.00±4.58^{ab}	20.67±4.93^{b}
GA only	6.17±0.15^{a}	2.53±0.87^{ab}	3.63±0.81^{a}	1.73±0.55^{ab}	14.66±3.79^{ab}	15.00±2.65^{ab}
OG only	5.77+0.06^{a}	1.86+0.78^{b}	3.90+0.72^{a}	2.57+1.85^{b}	14.00+5.29^{ab}	12.66+5.69^{a}

- Values along each column having different superscript letter (s) were significantly different ($p < 0.05$).
Values are mean±SD

*CN - Cyanide only
*CN+GA – cyanide and*Gnetum africanum* extract only
*CN+OG – cyanide and*Ocimumgratissimum*extract only
*GA – *Gnetum africanum* extract only
*OG –*Ocimumgratissimum*extract only

Table 4.4: Concentration of kidney function parameters after 14-day treatment

Treatment group	BUN (mg/dl)	Creatinine (mg/dl)
Control	2.67±0.58 [a]	0.60±0.63 [b]
CN only	1.67±1.16 [a]	1.10±0.10 [ab]
CN+GA	2.33±0.58 [a]	1.37±0.06 [a]
CN+OG	1.20±0.72 [a]	0.73±0.21 [bc]
GA only	2.00±1.00 [a]	1.23±0.21 [ac]
OG only	1.93+1.10 [a]	1.20+0.20 [ac]

- Values along each column having different superscript letter (s) were significantly different ($p < 0.05$).
Values are mean±SD

*CN - Cyanide only
*CN+GA – cyanide and*Gnetum africanum* extract only
*CN+OG – cyanide and*Ocimumgratissimum*extract only
*GA – *Gnetum africanum* extract only
*OG –*Ocimumgratissimum*extract only

CHAPTER FIVE

5.0 DISCUSSION

There were no mortalities recorded throughout the study. This was similarly observed in a 13-week cyanide toxicity study of sodium cyanide in drinking water of mice byNTP, 1993. The principal detoxification pathway of cyanide tothiocyanate in the presence of sulfur donor, mainly catalyzed by a liver mitochondrial enzyme, rhodanese (Tylleskar *et al*, 1991) could be responsible for the survival recorded in all rats. The morbidity or mortality depends upon the magnitude of poisoning, which varies with the dose, time of exposure, form of cyanide and the route of poisoning (Marrs *et al*., 1996; Hall and Rumack, 1986).

Several studies have shown that chronic cyanideexposure resulted in reduced body weight and lowerweight gains in several species including broilers(Panigrahi *et al*., 1992), rats (Sousa *et al*., 2002), dogs(Ibebunjo *et al*., 1992), pigs (Tewe *et al*., 1984), sheep(Onwuka *et al*., 1992) and goats (Soto-Blanco *et al*.,2001a).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 LD_{50}) for 14 days was assessed. Sub-acute exposure of KCN did not produce any significant change in body weight of the animals.In the present study, average body weight showed no significant difference(Table 4.1), although drastic reduction in body weight gain lower than the control group was observed within the last five days of the study (Figure 4.1). Considerable contrast to this observation was noted in*Gnetum africanum* only group (Figure 5)

Three hypotheses by Soto-Blanco*et al*., 2008have been proposed toexplain the negative effects of cyanide on weight gains:

(1) Depletion of the sulphur-containing amino acidscaused by their use as a source of sulphur for cyanide detoxification(Swenne *et al*., 1996).

(2) Impaired secretion of growth hormoneand reduced number of growth hormone receptorssecondary to hypothyroidism (Soto-Blanco *et al*., 2001a)and

(3) Impaired cellular energy metabolism mediatedby cyanide inhibition of mitochondrial oxidative phosphorylation(Sousa *et al*., 2002).

Animal weight gain, feed and water consumption wereaffected by *Gnetum africanum* and *Ocimum gratissimum* treatmentin this study. Decreased body weight change was observed in cyanide treated group (Figure 4.1). This could be attributed to inadequate dietary intake, loss of appetite and palatability facilitating the observed decrease. The deceased body weight change is as a result of the animals using the sulfur-containing amino acids of their body to detoxify cyanide, since their dietary sulfur-containing amino acids, being insufficient could not support the detoxification. During adaptation to a low protein intake, sulfur is conserved but cyanide detoxification is still possible at the cost of extensive protein catabolism. It is thus possible that subclinical cyanide exposure could interfere with normal growth and development.

Gnetum africanum significantly increased the body weight of the rats than other treatments when compared with the control as indicated in Table 4.1.The increase in weight may be associated with high quality nutrientscommonly found in the leaves of *Gnetum africanum* (Fokou and Domngang, 1989; Mialoundama, 1993), particularly its rich fat content, significant up to 14.20% (Okafor *et al*., 1996).Saponins are known to be toxic to body systems(Watt and Breyer-Brandwijk, 1962). In a study conducted by Effraim *et al.*, 2000, the findings revealed reduction in feed intake and loss of weight in all rabbits administered with *Ocimum gratissimum*extract.Similarly, in the present study, reduction in body weight was recorded in groups administered with *Ocimum gratissimum* (Table 4.1). According to Effraim *et al.*, 2000, the effect of saponins may have contributed to the clinicalsigns of loss of appetite (as deduced by reduction in feed intake) and loss of weight observed in allrabbits administered with the extract.

The early symptoms of cyanide poisoning include anxiety and excitement, weakness, headache, nausea, vomiting, drowsiness, dizziness, irritation of the eyes, nose and throat, and rapid breathing(Bhattacharya,2000; Baskin and Brewer, 2006). Cyanide can cause blindness and damage to the optic nerves and retina **(**NTP, 1993; ATSDR, 1997). Local effects of cyanides on the eye have been demonstrated to include conjunctival hyperaemia with mild chemosis, lacrimation, photophobia, and tingling sensation (IPCS, 1992).

According to Baskin and Brewer, 2006, cyanide produces irritation of the eyes and mucous membranes similar to that produced by riot control agents. In this study, nasal discharge and ocular lesion were observed in cyanide toxicity which were not annulled by

Ocimum gratissimum. However, *Gnetum africanum* cancelled the effects of cyanide on nasal discharge and ocular lesion when combined with cyanide (Group 3) as shown in figure 6 and 7. Poor dietary protein utilisation could be responsible for the manifestation of these local effects. According to Umoh *et al.* (1986), dietary protein deficiency prolongs the time of metabolism and hence increases the toxicity of cyanogenic glycosides in the body.

Vegetables are rich sources of carotene, ascorbic acid, riboflavin, folic acid, and minerals like calcium, iron and phosphorus (Fasuyi, 2006). In addition, they contain antinutrientswhich reduce their bioavailability (Akindahunsi and Salawu, 2005). Result of this study shows that the treatments administered at the concentration used and for the duration of the experiment appeared to adversely affect the haematological parameters as compared with the control (Table4.2). There was reduction in average quantity of red blood cell, haemoglobin and packed cell volume values in the groups treated with*Gnetum africanum*, *Ocimum gratissimum* and cyanide when compared with the control.Cyanide-treated groups significantly reduced the red blood cell counts, haemoglobin, and packed cell volume values. However, the reduction in red blood cell counts was not significant in*Gnetum africanum*-treated groups (3 and 5) and *Ocimum gratissimum* only (group 6). Cyanide causescellular hypoxia and cytotoxic anoxia resulting in tissue damage, throughout the body, with the most vulnerable tissues being those with high oxygen demands and/or a deficiency in detoxifying enzymes such as rhodanese (Guatelli, 1964; Ballantyne, 1974; Egekeze and Oehme, 1980). The reduction noted in cyanide treatment groups may have resulted due to lysis of the blood cells due to the absence of oxygen in the blood cells resulting in a defect in the haemoglobin molecule itself. In a study conducted by Iweala and Osundiya, 2010, groups of rat fed with *Gnetum africanum*-supplemented diet had reduced packed cell volumelevel. Similarly, this research recorded significant reduction inpacked cell volumelevel, and consequently, inred blood cell and haemoglobin of rats fed with the extract. However, the decrease in the red blood cell counts was not significant.The liver has an important role in the regeneration of red blood cell. According to Eissa and Zidan, 2009,red blood cell counts can be reduced due to the failure of the liver to supply the blood circulation with cells from haemohepatic tissues, and the possible destructive effect on red blood cell bythe antinutrient factors.Since PCV levels reflect the extent and efficiency of oxygen uptake and transfer to tissues, (Ots *et al.*, 1998), the low values in the treatment groups may reflect low oxygen uptake and

transfer to tissues, signifying a reduction in the body's metabolic activity (Carpenter, 1975). *Gnetum africanum* only had the lowest haemoglobin and packed cell volumevalues compared to other treatment groups.The reduction of red blood cell counts noted in *Gnetum africanum* treatment groups could be attributed to its phytochemical constituents.

In a study conducted by Effraim *et al.*, 2000, rabbits exposed to *Ocimum gratissimum* had reduction in the red blood cell, haemoglobin and packed cell volume. The result in this study concurs with the findings of Effraim *et al.*, 2000 on the red blood cell and packed cell volume level. However, contrary to their findings, the haemoglobin level remained unchanged. *Ocimumgratissimum*combined with cyanide group showed significant reduction in haemoglobin,red blood cell and packed cell volumelevels, but the reduction in the red blood cell and packed cell volumelevels observed in *Ocimum gratissimum* only group, was not significant. This observation is at variance with the local ethnotherapy of ingesting decoction of leaves of *O. gratissimum* as blood tonic in some parts of Nigeria as haematinic nutrients.

Reduction in red blood cell, haemoglobin and packed cell volumeby*Gnetum africanum*- and *Ocimum gratissimum*- containing treatments probably occurred due to suppression of the blood cell synthesis by saponin found in the leaf extracts (Irvine, 1961). The significant reduction by*Gnetum africanum* than the*Ocimum gratissimum* treatment in comparison with the control could be, in addition to saponin content, the presence of greater cyanogenic glycoside level observed by Iwala*et al.*, 2009.

In this study, white blood cell counts was increased with the consumption of *Gnetum africanum, Ocimum gratissimum* and cyanide treatments when compared with the control (Table4.2).The level of white blood cells (WBC) was used as an index of immune function. The increase in the level of white blood cells recorded in this study is in line with the work done on *Gnetum africanum* by Iweala *et al.* (2009). According to the findings of Iweala and Obidoa, 2010, *Ocimum gratissimum* increased the WBC of rats fed with *Ocimum gratissimum* diet. WBCs are involved in the cellular and humoral defense of the organism against foreign material (Jimoh*et al*., 2008). Increase in the level of white blood cellsis common when there is prolonged exposure to the potentially toxic agent or persistence of the injurious agent (Aster and Kumar, 1999). However, the effect

of *Gnetum africanum*, *Ocimum gratissimum* and cyanide treatments on mean white blood cell counts is not significant indicating a possible "no effect" on the integrity of white blood cells.

Platelets play major role in controlling haemorrhage which can be summarized as primary aggregation, secondary aggregation, blood coagulation, clot retraction and clot removal (Janqueira and Carneiro, 2005). Platelet concentration as well as PCV are determinants of blood viscosity, which correlates positively to blood pressure (Sharp *et al*., 1996;Ho, 2004). Result of these study revealed that *Gnetum africanum, Ocimum gratissimum* and cyanide treatments had no significant effect on blood platelet in rat blood (Table 4.1). However, *Gnetum africanum* only treatment had the highest platelet count while *Ocimum gratissimum* only had the least count when compared with all the treatment groups. The decrease in platelet values of rats administered with *Ocimum gratissimum* only noted in this study was similar to the findings of Jimoh *et al*., 2008 and Effraim *et al*., 2000.Increase in platelet counts could depict signs of haemorrhage which triggered more production of blood platelet to control bleeding. However,the increase noted in cyanide only, *Gnetum africanum* only, *Ocimum gratissimum* and cyanide only groups were not significant, implying that platelet level in the blood was not compromised.

Lymphocyteslevel decrease is frequently noted in the initial stages of invasion of foreignmaterials indicating diseases that affect the immune system. An increase in the number of lymphocytes is usually noted in prolonged illnesses. In this study, decrease inlymphocyteslevel was noted in cyanide only and*Gnetum africanum* administered treament groups.*Gnetum africanum* treatment groups (groups 3 and 5) showed more decrease in lymphocyte production, especially group 3; this could result from a more threatening invasion destructing lymphocyte cells in the presence of poor diet intake (Table 4.1). However, antioxidant phytochemicals, like flavonoids, are known to protect lymphocytes and reduce their destruction (Duthie *et al*., 1996).In contrast, an increase inlymphocyteslevel was observed in*Ocimum gratissimum*administered treament groups. However, these changes did not show any substantial differences from controls.

In this study, neutrophil level was increased in all the treatment groups except in*Ocimum gratissimum*only group when compared with the control (Table 4.1). The gradual increases in neutrophil counts across the intervals in all treated groups reflect a stressful

condition impacted on the immune system (Mbajiorgu *et al*., 2007). However, these changes were not significant implying that all the treatments did not elicit any negative effect on neutrophil level.

Monocytes play critical roles in innate and adaptive immunity during inflammation (Burdo *et al*., 2010). Result of the present study revealed a very low count of monocyte cells (Table 4.1), which could result from immunosuppression(Fingerle-Rowson *et al*., 1998) and bone marrow failure and/ or damage. Exact reasons of decreased monocyte counts noted in all the treatment groups, especially *Ocimum gratissimum* only group, are not yet clearly understood, however, the decrease could be due to inhibition of haematopoiesis, increased rate of destruction of monocyte cells or a combination of both.

Result of the present study shows that administration of *Gnetum africanum*only increased the level of eosinophil count than the normal (Table 4.1). Eosinophils are often elevated with allergic reactions (Fischbach, 1996). In contrast, *Ocimum gratissimum* and cyanide treatments decreased eosinophil level. However, these changes had no significant effect on eosinophil cells.

Table 4.2shows that there was significant reduction in total protein by all cyanide-containing treatments. The decrease of total serum protein could have been necessitated by the reduction of serum globulin level which markedly declined at the same time (Zama *et al*., 2005). Cyanide is converted to thiocyanate (SCN^-), a reaction that requires sulphane sulphur as a rate-limiting cofactor for the enzyme rhondanese (Lundquist, 1992). The concentration of sulfane sulphur is dependent on the availability of sulphur amino acids from dietary protein (Cliff *et al*., 1985). Even in protein malnutrition, available sulphur is preferentially utilized for cyanide detoxication (Swenne *et al*., 1996). The preferential use of metabolically available sulphur-containing amino acids for cyanide detoxification in the bodywhere dietary intake of protein is inadequate, could be a responsible factor for the decrease in protein level in these groups.

Proteins play an important role in the life of all living organism (Afshar *et al*., 2008). Cyanide disturbs protein synthesis (Okafor *et al*., 2008; Dogan *et al*, 2006;Scott and Weir, 1981; James *et al*, 1994). A similar decrease of total protein concentration was recorded in serum of rats that were treated with cyanide by Okafor *et al*., 2008. The weight gain verified during the experimental period noted in group 3 (*Gnetum africanum*

and cyanide) (Table 4.1 and Figure 4.2) was due primarily to fat disposition. Several studies have shown thatphytates and tannins inhibit thebioavailability of proteins and minerals (Davidson *et al*.,1975). Tannins are capable of loweringavailable protein and minerals by antagonistic competition and can therefore elicitprotein deficiency syndrome, kwarshiorkor while phyticacid has complicated effect in human system includingindigestion of food and flatulence (Maynard, 1997).These may explain reduction of total proteins found in rats (groups 3 and 4) treated with cyanide.

Plant foods such as *Gnetum africanum* have both nutrient and non-nutrient components (Isong *et al*., 1999; Iwu, 1986). These nutrients especially proteins, carbohydrates and lipids are needed for growth, body repair and maintenance (Davidson *et al*., 1975). The leaves of *Gnetum africanum* constitute an important source of protein, essential amino acids and mineral elements (Ouabonzi *et al*., 1983).Thisquality of *Gnetum africanum*is reflected in the group treated with *Gnetum africanum*only. There was increase in total protein level and albumin which constitutes the total protein in living system in the treated rats as indicated in Table 4.3. The protein level noted in *Gnetum africanum* only and *Ocimum gratissimum* only appeared normal from the reference value which could imply adequate utilisation of dietary protein, hence, no compromise on the serum protein of the rats.

One of the obvious implications of utilization of the sulfur amino acids for detoxification of dietary cyanide under low protein intake is that protein synthesis is compromised. This is clearly seen in decreased body weight (Figure 4.1) and very low serum globulin level (Table 4.2) of the cyanide treatment group compared to the control. Despite this, the rats did not suffer a marked decrease in their albumin levels, possibly because of poor utilisation of dietary protein by the liver, which may be responsible for the elevation in levels of albumin (Table 4.2) which exhibits enzymelike behavior and uses bound elemental sulfur (Sykes, 1981) to detoxify cyanide(Baskin and Brewer, 2006) in the cyanide-exposed groups. Cyanide may be sequestered by albumin and metabolized to 2-aminothiazoline- 4- carboxylic acid (ATC) (Bitner *et al*., 1995, 1997; Lundquist *et al*., 1995) or to cyanate (OCN^-) which (Swenne *et al*., 1996), in turn, is converted by the cysteine- containing enzyme cyanase [E.C. 3.5.5.3] to ammonia and bicarbonate (Schultz, 1984).These activities could have necessitated the increased production of albumin in groups 2, 3, and 4. However, the result presents that the levels of albumin production

induced by cyanide,*Ocimum gratissimum* and *Gnetum africanum*treatments are within normal range.

This study revealed a significant low levels of globulin in groups 2. 3 and 4. Globulins, especially gamma-globulin which are the commonest, are antibodies. Low globulin level in the blood impliesantibody deficiency. The abnormality in the values for globulin and monocyte count suggests nutritional deficiency which led to immune depression. Serum globulin level may be decreased in Nephrosis as earlier discussed.However, *Ocimum gratissimum*only and *Gnetum africanum* only showed no effect on globulin levels.Antibodies are produced by mature B lymphocytes. The antioxidant phytochemicals such as flavonoids which modulate immune function by protecting lymphocytes (Duthie *et al*., 1996) could alsobe responsible for this non significant effectthe on globulin levels in the absence of nutritional deficiency.

Albumin/globulin ratio suggested that *Ocimum gratissimum* and *Gnetum africanum*treatments appeared to be hepatoprotective(ratio 1.73 $\leq$2.2) in this study (Table 4.3).Although albumin is made exclusively in the liver, globulins are produced in many sites throughout the body. Thus, whether total protein is normal, elevated, or low, a decrease in the A/G ratio often indicates the presence of impaired liver function. It is also possible that the extracts exhibited hepatoprotective activity due to their antioxidant property attributable to bioavailable flavonoids which act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes (Ephraim *et al*., 2000; Iweala *et al*., 2009;Robak and Gryglewski, 1988).However, cyanide-containing treatment groups showed decreased A/G ratio, though not significant, implying a disturbance in the normal liver function which was annulled in time to abate the negative effects of these treatments on the liver.

The liver is the key of metabolism, secretion and excretion and it is continuously and variedly exposed to xenobiotics,environmental pollutants, and chemotherapeutic agents because of its strategic location in the body.Cyanide toxicity is also caused by increased generation of superoxide anion and lipid peroxidation (Daya *et al.*, 2002) with inhibition of antioxidants enzymes (Ardelt *et al*, 1994).Liver enzymes (AST and ALT) are liberated into the blood whenever liver cells are damaged and enzyme activity is increased in the plasma (Edward *et al.*, 1995). Result of this study presents reduction in AST level in

groups of rats treated with cyanide, *Gnetum africanum* and *Ocimum gratissimum* extracts. There was a significant reduction in AST level of rats in Group 3. The significantly lower AST scores in the treated animals suggests that there may be a reduction in inflammatory processes when rats are consuming cyanide with *Gnetum africanum*. However, the reduction noted in groups 2, 4, 5 and 6 were not significant. It has been suggested that the development of syndromes is dependent on the rate and duration of exposure to cyanide in tight conjunction with the capacity for cyanide detoxification (Tylleskar *et al.,* 1992). The observation on the present study agrees with the fact that the system has the recuperative ability to annul a posing threat to its function within its capability.Aletor and Adeogun reported that some antinutritional phytochemicals exhibit protective effects against a number of biochemical, physiological and metabolic disordersin the body (Aletor and Adeogun 1995).This may explain a lower decrease in AST level of rats treated with *Gnetum africanum* and *Ocimum gratissimum* extracts. The fact that the levels of this liver enzyme were reduced indicates that the treatments, cyanide,*Gnetum africanum* and *Ocimum gratissimum*,did not have necrotic effect on the liver.

On the contrary, ALT levels were elevated in cyanide-treated groups of rats, except in Group 3 which slightly reduced these levels when compared with the control, though these changes were not significant. The elevation of ALT levels could imply the signs of impending leakage from the liver into the blood stream when administered in rats as earlier discussed during the inflammatory process. However, these effects exerted by cyanide is not significant as explicated by the changes in A/G ratio. The result presents that*Gnetum africanum* only and *Ocimum gratissimum*only had no such inflammatory effects on the liver.

Uric acid and creatinine are useful in early deduction of nephrotoxicity induced by exogenous compounds (Eissa and Zidan, 2009). After protein metabolism, urea is the waste product released, which is excreted with urine but the nitrogen contents of the urea are sometimes found in the blood stream as a result of kidney malfunctioning (ATSDR, 1997). The result in this study presents a non significant decrease in blood urea nitrogen (BUN) in all the groups when compared with the control. One of the causes of decreased concentration of BUN is low protein diet. Dietary protein intake can modulate renal function (King and Levey, 1993). This implies that lower protein intake would have a longer time to renal failure. Cyanide combined with*Ocimum gratissimum* (group 4) had

the least concentration of BUN reflectinglow protein dietary intake and poor protein utilisation induced by the antinutrient agents in the plant. Cyanide only (group 2) was next in line reflecting low protein dietary intake. Groups of rats treated with *Gnetum africanum* (groups 3 and 5) showed high concentration of BUN reflecting its rich protein content, with *Gnetum africanum* combined with cyanide (group 3) being the highest when the control group is not taken into consideration. High protein intake has the ability to promote renal damage by chronically increasing glomerular pressure and hyperfiltration (Martin *et al*., 2005).It has been established in a study that increased protein intake elevated rates of creatinine and urea excretion in dog model (Jollife and Smith, 1931). The common mechanism underlying increased excretion rates was eventually attributed to changes in glomerular filtration rate (GFR) (Shannon *et al*., 1932; Herrin *et al*., 1937) and van Slyke *et al*., 1934, demonstrated that renal blood flow was the basis for GFR mediated changes in clearance rates in response to increased protein intake. Increased consumption of dietary protein is linearly related to the production of urea (Young *et al*., 2000) and urea excretion is controlled by the kidney. Increased protein intakes has been linked with increased water requirement necessary for excretion of urea nitrogen (Martin *et al*., 2005). This could explain the increased water consumption in *Gnetum africanum* treated groups observed in this study. However, the study revealed that the changes in blood urea nitrogen did not have any biological significant effect on the rats.

Creatinine levels were elevated in the treated rats when compared with the control as indicated in Table 4.4. An elevated creatinine is a more indication of impaired kidney function than BUN. This is because creatinine is affected minimally by liver function unlike BUN.

Creatinine, a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the muscle mass of an individual. In a 40-week study of rabbitson potassium cyanide at a level of 1.76 g/kg diet (corresponding to 24–17 mg cyanide/kg body weight per day), creatinine levels were elevated (Okolie & Osagie, 1999). Creatinine levels has been implicated in dietary protein malnutrition. Protein supplementation improves muscle strength (Bonjour, 2011). Diets deficient in calories and/or protein can cause loss of weight and muscle mass (Kuizon and Salusky, 1997). Causes of malnutrition include anorexia induced by mechanisms linked to accumulated toxins (Anderstam *et al*., 1996) and abnormal protein and amino acid metabolism (Meireles *et al*., 1999). This could explain the elevation noted in cyanide only and

Ocimum gratissimum-containing treatments due to malnutrition (Table 4.1 and 4.4). There were increase in creatinine levels in groups treated *Gnetum africanum* when compared with the control. More importantly, is the significant increase when *Gnetum africanum* is combined with cyanide (group 3). This supports the earlier observation of the possibility of this combination resulting in kidney impairment. The adverse effects on the kidney could have resulted from stress on the kidney due to high dietary protein in a compromised immune system referable to malnutitrition (Table 4.1, 4.2, 4.3 and 4.4). Elevation of creatinine concentration in serum of treated rats indicates diminished ability of the kidneys to filter the waste product from the blood and excrete them in the urine reflecting dysfunction of the kidney tubules (Hayes, 1989; Walmsley and White, 1994).This result explains that immoderate dietary protein intake may result in kidney dysfunction.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

Oral administration of cyanide-containingtreatments at the concentration used onrats produced the symptoms of toxicity due to cyanideexposure.

Co-administration of cyanide with *Gnetum africanum*suppressed the haemopoietic system and did not alleviate the haematological and the biochemical effects of cyanide toxicity.

The administration of *Ocimum gratissimum*alone did not have any biological significant effect on haematological and biochemical indicators, except on the kidney, where there was elevation in creatinine level,suggesting its potential to initiate or promote renal disease. However, *Gnetum africanum* had more deleterious effects.

Subchronic consumption of these plants, especially *Gnetum africanum*, with cyanoglycoside-containing food is not recommended.

Furtherresearches should consider the activity guided fractionation analysis of these plants forthe identification of the components (molecules) responsible for thesuppression of blood parameters. Future studies will also attempt to determine the probable mechanism of action of the vegetables.

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