EFFECTS OF VITAMIN C ON SOME HAEMATOLOGICAL PARAMETERS AND BIOMARKERS OF OXIDATIVE STRESS IN ALBINO WISTAR RATS EXPOSED TO SHORT-TERM LEAD ACETATE

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Declaration

I hereby declare that the work in this thesis entitled **EFFECTS OF VITAMIN C ON SOME HAEMATOLOGICAL PARAMETERS AND BIOMARKERS OF OXIDATIVE STRESS IN ALBINO WISTAR RATS EXPOSED TO SHORT-TERM LEAD ACETATE**was performed by me in the Department of Human Physiology under the supervision of Dr. A Mohammed and Dr Y. Tanko

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma in any institution.

HASSAN NANA AISHAH …………………… ………………
Name of Student SignatureDate

Certification

This dissertation entitled **EFFECTS OF VITAMIN C ON SOME HAEMATOLOGICAL PARAMETERS AND BIOMARKERS OF OXIDATIVE STRESS IN ALBINO WISTAR RATS EXPOSED TO SHORT-TERM LEAD ACETATEby Hassan Nana Aishah** meets the regulations governing the award of the degree of Master of Science (Human Physioology) of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation**by Hassan Nana Aishah** meets the regulations governing the award of the degree of Master of Science (Human Physioology) of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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Dedication

To Almighty Allah for giving me wisdom, sound health, favour and life. To Him alone belong all the glory, adorations, worship and praises.

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Abstract

The effects of vitamin C on some heamatological parameters and biomarkers of oxidative stress in albino wistar rats exposed to lead acetate over a short term (3 weeks) was investigated. Studies have revealed that lead has a wide range of health effects that can result from exposure, and that lead can cause health effects at blood lead levels previously thought to be safe. An increasing body of evidence suggests that lead is associated with a number of health conditions. Twenty albino wistar rats were randomly divided into four experimental groups of five rats each, Control group were fed normal rat feed with distilled water, Group 2,3 and 4 were fed normal rat feed, water and received daily oral administration of lead acetate 250mg/kg daily. In addition groups 3 and 4 received 100mg/kg and 150mg/kg oral administration of vitamin C respectively, for three weeks. Mild effect of lead acetate was observed in haematological parameters as indicated by slightly increase ,which was not statistically signifiant, in RBC and MPV. In contrast, a slight decrease was seen in haemoglobin, PCV, MCV, MCH, MCHC and platelet count. Descrease in MCV,MCH and MCHC indicated shrink in size of RBCs and onset of microcytic anemia due to onset of iron deficiency. However, derease observed was not stgatistically signifiant. Also the effect of lead acetate was mild on biochemical enzyme activities, indicated byincreased level of MDA activity; whih indicate oxidative stress,however the increase was not statistically significant. SOD and GPx level were slightly decreased, which was not statistially signifiant. The doses of vitamin C supplement did not reverse the effect of lead acetate on some haematological parameters and some biochemical enzyme activities, it however reverse the effect of lead acetate on MDA level. In conclusion, exposure to lead acetate over a short term has little effect on haematological parameters and biochemical enzyme activities. The doses of vitamin C use in this study has ameliorative effect on MDA activity but has no effect on some alteration on haematological parameters and some biochemical enzyme activities.

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List of abbreviations

ALA- δ-aminolevulinic acid

ALAD - δ-aminolevulinic acid dehydratase

ALAS -δ-aminolevulinic synthetase

ALAU- Urinary δ-aminolevulinic acid

ApoE- Apolipoprotein E

ATP Adenosine triphosphate

BBB - Blood brain barrier

DMTI- Divalent metal transporter

EDTA mobilization,

EP - Eythrocyte protoporphyrin

EPA - Environmental Protection Agency , U.S.

GFR - Glomerular filtration rate

HDL - High density lipoprotein

HHS - Health and Human Services

IQ - Intelligence quotient

NHANES - The National Health and nutrition examination survey

PAD - peripheral arterial disease

PbB – Blood lead burden

RR - Risk ratio

TTP - Time-to-pregnancy

CHAPTER ONE

1.0 INTRODUCTION

1.1 GENERAL INTRODUCTION

Lead is a naturally occurring bluish-gray heavy metal found in small amounts in the earth's crust. However, it is rarely found naturally as a metal. It is usually found combined with two or more other elements to form lead compounds (ATSDR, 2007). Metallic lead is resistant to corrosion (i.e., not easily attacked by air or water). When exposed to air or water, thin films of lead compounds are formed that protect the metal from further attack (ATSDR, 2007). Lead is poisonous when inhaled or eaten. Lead content in air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries (Tuarmaa, 1995). According to WHO (2000) lead is a metal with no known biological benefit to humans. Too much lead can damage various systems of the body including the nervous and reproductive systems and the kidney.

Generally, heavy metals produce their toxicity by forming complexes or ligands with organic compounds thereby affecting the function of biological molecules, inactivate some biochemical enzymes and affect protein structure (Pirkle, 1998) Because of its potential health problems, the amount of lead used in these products today has lessened or has been removed. Lead and other heavy metals create reactive radicals which damage cell structure including DNA and cell membrane (Flora, 2008). Lead poisoning can cause a variety of symptoms and signs which vary depending on the individual and the duration of lead exposure (Kosnett, 2005, Karri, 2008).

The amount of lead in blood and tissues, as well as the time course of exposure, determines the level of toxicity (Pearson and Schonfeld, 2003).

The absorbed lead enters the blood stream where over 90 percent of it is bound to the red cells with a biological half life of 25-28 days (Az*ar*, 1975). Toicological effects of lead have their origin in perturbation in cell function of various organ systems. The major biochemical effect of lead is its interference with heme synthesis which leads to haematological damage (Awad and William, 1997). Impaired synthesis of heme interfers with oxygen transportation to the tissue. Lead interferes with the production of heme at several different steps. Lead exposed persons can develop anaemia. In adults, anaemia is usually seen in severe chronic lead poisoning and blood lead levels of 70 µg/dL and higher are usually found (Gulson and Salome, 1996). Animal models exposed to lead show significant changes in haematological parameters (Ashour, 2006) Gulson and Salome (1996) stated that lead is a cumulative poison. Unlike acute poisons, such as chemicals that can kill quickly by attacking the lungs, lead poisoning happens slowly. The lead that is taken in daily, mounts up in the tissues, especially the bones. Blood lead levels mainly show recent exposure, however; lead that is removed from bone is also present in the blood. It is quite possible for higher amount of lead in the body than the blood lead level.

Vitamin C is a water-soluble micronutrient required for multiple biological functions (Halliwell, 2001). It is found intra- and extracellularly as ascorbate, and is well absorbed from the gastrointestinal tract (Chihuailaf, 2002; Woollard, 2002; Asiley, 2004). Vitamin C is a natural antioxidant that prevents the increase production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Sies,1992).

The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may particularly prevent certain types of hepatic cellular damage (McDowell, 1989; Parola, 1992; Sies, 1992; Burtis and Ashwood, 1994).According to Patrick, (2006a) consumption of 100 mg of vitamin C a day has been shown to significantly decrease lead levels in some, though not all, cases - apparently more through reduced absorption rather than increased excretion. Vitamin C has been consistently shown to protect the concentration of molecules such as ALA dehydratase that are associated with red blood cell manufacture. Vitamin C improves iron absorption; it can mix with food in the stomach, as well as increasing iron's capacity to displace lead during food absorption. There is some evidence that Vitamin C can inhibit lead uptake at a cellular level as well as lead's cellular toxicity. Experiments with rats have demonstrated reduced lead impacts on a variety of body organs.

1.2STATEMENT OF RESEARCH PROBLEM

Lead exposure remains one of the most important problems in terms of prevalence of exposure and public health impact (Hu *et al*., 2006). Worldwide, lead poisoning accounts for nearly 1% of the global burden of disease (Landrigan and Ayuso-Mateos, 2004). Despite decades of intensive research, lead poisoning remains one of the most, if not the most, studied subjects of all within the fields of environmental health and environmental medicine (Hu *et al*., 2006).Lead exposure occurs via four basic media: food, dust or dirt/soil, air and water and mostly via two routes, inhalation and ingestion. Mushak and Crocetti (1996) have reported on the large body of literature showing that deficiencies or alterations in essential nutrients like calcium, iron, phosphorus and zinc enhance lead exposure and increase the degree of lead toxicity associated with such exposure.

Cells absorb lead through the same channels they absorb calcium from. The drugs that regulate the intake of calcium also increase the amount of lead uptake. High levels of lead decrease transport of calcium and vice versa, therefore these two metals function as competitive inhibitors. Lead can enter through the same ion channels as calcium and regulate the activity of those channels to uptake more lead into the cell (Patel, 2000).

Epidemiological studies and clinical observations provide evidence for a progression of adverse health effects of lead in humans that occur in association with blood lead ranging from <10 to >60 μg/dL (ATSDR, 2007). Over the past century in particular, increasingly sophisticated epidemiological studies have more adequately revealed the wide range of health effects that can result from exposure to lead, and that lead can cause health effects at blood lead levels previously thought to be safe. An increasing body of evidence suggests that lead is associated with a number of health conditions (Schafer *et al*., 2005).

Lead has long been known to alter the hematological system. At the low end of the blood lead concentration range, adverse effects include increased blood pressure and inhibition of pathways in heme synthesis. The anemia induced by lead results primarily from both inhibition of heme synthesis and shortening of the erythrocyte lifespan (ATSDR, 2007). In most cases the symptoms of lead are misdiagnosed and people are unaware of the damage that can be caused by lead. It recent years more attention and research has been directed towards lead poisoning and its effect on the human body but little has been done to access its effect on hematological parametersand anti oxidadant emzyme activities.

1.3 JUSTIFICATION

There has been an increasing incidence of lead poisoning all over the world. Despite the attempt for reducing the exposure to this metal, there is still some report of cases of severe lead toxicity (Hershko, 2005; Roch , 2005; Coyle 2008) . According to Escribano (1997) 140 mg/kg dose was an approximate environmental daily-exposure level.The effects of low-level lead poisoning in children may be irreversible and there may be no threshold for health effects. Numerous risk factors predispose children to both higher exposure and greater vulnerability to the hazards of lead.

Children in low-income countries are usually at elevated risk of exposure to lead, involving multiple sources and higher levels of exposure than observed in high-income countries (Nriagu *et al.,* 1996). African children, in particular, may be at high risk of lead exposure and poisoning as a consequence of a paucity of information available to the public on the sources and mechanisms of exposure to lead in children, ongoing use of lead in many products, inadequate regulatory frameworks, weak enforcement of existing legislation, high levelsof poverty and inequity, poor housing conditions and extensive malnutrition (Nriagu *et al*., 1996; Tong *et al*., 2000).

Studies conducted at mines around the world have shown that children living in close proximity to a mine may be at increased risk of lead exposure (Lyle *et al*., 2006; von Schirnding *et al*., 2003).More than 400 children have died in five villages, most of them under three years of age from lead poisoning in Zamfara state, Nigeria (Grossman, 2012),

According to WBG, (1996) lead is found frequently in our environment and in our body, it has no known purpose in the body. In developing nations, massive lead contamination is occurring from the continued use of leaded gasoline. Lead levels along roads in Nigeria approach 7000 parts per million, about 15 times higher than the level used to designate a toxic Superfund Site in the U.S. In Mexico City, half the children tested have dangerous levels of lead in their blood and in Cairo, more than 300 infants die annually due to maternal lead exposure(WBG, 1996).

With the wide occurrence of lead poisoning in both developed and developing nations from food, drinks and contaminated soil, it is therefore necessary to assess the effect of lead poisoning on haematological parameters and biomarkers of oxidative stress and assess the beneficial effect of Vitamin C in reversing the effect of lead on haematological parameters and biomarkers of oxidative stress since they are used to measure physiological disturbance and level of damage cause by disease or toxic substance to the body.

1.4AIM

The aim of this study is to investigate effect of vitamin C on some haematological parameters and biomarkers of oxidative stress in albino Wistar rats exposed to short term lead acetate.

1.5 OBJETIVES

i. To evaluate the effect of short-term vitamin C-lead acetate exposure on haematological parameters such as RBC, ,Hb, MCV, MCH, MCHC, MPV,platelets count in albino Wistar rats

ii. Toevaluatethe effect of short-term vitamin C-leadacetate exposure on MDA, SOD and GPxactivity in albino Wistar rats.

1.6NULLHYPOTHESIS

Vitamin C has no effect on haematological parameters and biomarkers of oxidative stress in albino wistar rats exposure to lead acetate.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1INTRODUCTION

In the modern society, thousands of hazardous chemicals and heavy metals are being produced and used in a wide variety of work places all over the world. Heavy metals are trace metals that are at least five times denser than water and are taken into body via inhalation, ingestion and skin absorption. It should be noted that most of the pathological conditions in body arise as a result of the exposure to these injurious substances(Barber *et al.*, 2011).

Lead is the most abundant of the heavy metals in the Earth's crust A ubiquitous and versatile metal, it has been usedsince prehistoric times (WHO, 2000).Lead is undoubtedly one of the oldestoccupational toxins and evidence of lead poisoning canbe found dating back to Roman times. As industrial leadproduction started at least 5000 years ago, it is likely thatoutbreaks of lead poisoning occurred from this time (Gidlow, 2004). It has become widely distributed and mobilized in the environment, and human exposure to and uptake of this non-essential element have consequently increased (WHO, 2000).

Since it wasdiscovered thousands of years ago, these propertieshave resulted in lead being used in a myriad ofprocesses and products. However, there is now anoverwhelming body ofevidence associatingleadexposure with a wide range of detrimental healtheffects. The use of lead increased dramaticallyaround the time of the industrial revolution. (Von Schirnding *et al.*, 2001).

Before the industrial revolution, body lead burdens were more than 500 times lower than what they are today. Lead is a persistent metal, and does not readily get converted to less toxic forms in nature (Von Schirnding *et al.*, 2001)

2.2 PROPERTIES OF LEAD

Lead has an atomic number of 82, atomic weight, 207.19 and specific gravity, 11.34. Its melting point is 327.5^{o}c and boiling point of 1740^{o}Cat atmospheric pressure. Lead exists in the valence states of +2 and +4 and has four naturally occurring stable isotopes with atomic weights 208, 206, 207 , 204 (in order of abundance). It valence shell has four electrons. Nevertheless its usual valence state is +2 owing to readily ionizable two electron (Geraldine, 2006)

Elemental lead is an odorless, silver-bluish-grey metal that is insoluble in water (Budavari *et al.* 1996; Lide and Frederikse 1998; HSDB, 2009). It is soft, highly malleable, ductile, and a relatively poor conductor of electricity. It is resistant to corrosion but tarnishes upon exposure to air. It forms salts of both organic and inorganic compounds. Lead compounds may be divided between those compounds that are relatively soluble in water and those that are relatively insoluble in water. Inorganic salts such as lead sulphide and lead oxide have poor solubility in water, lead nitrate, lead chlorate and lead chloride have better solubility. The major soluble lead compounds are lead acetate, lead acetate trihydrate, lead chloride, lead nitrate, and lead subacetate; all are soluble in water, and lead acetate trihydrate is miscible with water. Lead acetate exists as colorless or white crystals, granules, or powders that are soluble in glycerol and slightly soluble in ethanol. Lead acetate trihydrate occurs as white crystals that are slightly soluble in ethanol and acetone. Lead chloride exists as a white crystalline powder that is insoluble in ethanol. Lead nitrate occurs as colourless or white crystals. The lead salts formed with organic acids are lead lactate, lead oxalate and lead acetate. Lead acetate has better solubility in water as to insoluble lead oxalate. Stable lead compound may be synthesized where lead is directly bond to a carbon atom and this includes tetra ethyl lead which are of importance in fuel activity (HSDB 2009).

2.3 USES OF LEAD

In worldwide metal use, lead ranks behind only iron, copper, aluminum, and zinc (Howe 1981). The utility of lead and lead compounds was discovered in prehistoric times. Lead has been used in plumbing and tableware since the time of the Roman Empire. Lead usage increased progressively with industrialization and rose dramatically with the widespread use of the automobile in the twentieth century (WHO, 2006).

Lead is widely used in many industrial and domestic activities because of its excellent physio-chemical properties, low cost and easy workability (Herman *et al.*, 2003). Lead has found major uses in pipes and plumbing, pigments and paints, gasoline additives, construction materials and lead–acid batteries (WHO, 2006). Its softness, high density, low melting point, high boiling point, apart from making it pliable, permits pipes to be bent and curved without necessitating many joints (Geraldine, 2006).Its largest use is in lead-acid storage batteries for motor vehicles and general industry. Lead metal also is commonly used for ammunition, cable covering, brass and bronze, bearing metals for machinery, and sheet lead (ATSDR 1999). The uses of lead in pipes, paints and gasoline additives have resulted in substantial introductions of lead into the environment and human exposure, and are being phased out in many countries (Geraldine, 2006).

The predominant use of lead is now in lead–acid batteries and, to a lesser extent, in construction materials and lead-based chemicals (WHO, 2006). Because of its low elasticity, high coefficient of thermal expansion and excellent antifriction properties, it has found chief and varied role in many engineering applications. It contains small concentration of copper, silver, bismuth and alkaline earth metals, which makes it hard and enhances the corrosion resistance and hence is widely used in the chemical industries (Geraldine, 2006). Owing to its high density and high opacity to gamma and X-ray energies lead is desired for x-ray protection (Weinstein, 2003). The high density of lead coupled with its ease for casting and its salvage value makes it the ideal counterweight material in machinery andequipment. Because of its malleability, lead can be easily cold rolled to form sheet and slabs. Sheet lead is used as a flooring material in electroplating and chemical plants (Geraldine, 2006)Lead also hold pigments well, it is used in many colored pigments, colored inks and paints.

Further apart from acting as a dispersing and dying agent it provides durability to paints (Lovoie and Bailey, 2004)Lead glaze are used on ceramic kitchenwares, earthenware and stone ware because they allow more flexibility in the kiln at high temperature for firing pottery (Kuruvillo *et al*., 2004)

Lead uses also includeammunition, welding, solder, bullet, preparation of some brass and bronze products and foiul on the bottles of wine. It is also used in cosmetic products and in kohl in some Asian countries (CDC, 1991)

2.4HISTORICAL ASPECT

Lead is linked to the very beginning of mankind and its origin traced back to antiquity. Lead mining probably predates the iron or bronze ages (Geraldine, 2006) Lead was among the first seven metals discovered by humans. Some of these metals are termed “found” metals because they are stable upon surface of the earth. “Found” metals include the “noble” or coinage metals found near the end of the d block of the periodic table as found (Fitch, 2004). The earliest record of lead mine in Turkey. The oldest artifact of smelted lead is a necklace found in an ancient city site of Anatolia, the estimated age of the necklace being 6000 to 8000 years (Geraldine, 2006)The earliest written accounts of lead toxicity have been found in Egyptian papyrus scrolls.

According to them, lead compounds were often used for homicidal purposes. Hippocrates, in 370 BC, was probably the first to describe lead colic, without however recognizing the etiology. The first to describe lead palsy was Nicander in the 2nd century BC, but he too was not able to attribute the palsy to lead exposure. But in the 1st century AD Dioscorides saw the connection between lead exposure and toxic manifestations, and Pliny stated that lead poisoning was common in shipbuilding (Hernberg, 2000).

Lead was certainly one of the first metals to be mined by man in the known major civilizations (Winder, 1994). Lead is found with silver. Because of its existence with silver it was extensively mined (Fitch, 2004). Lead mines were worked in Sardinia, Athens, Carthage and Spain around 2000 BC and these workings were taken over by Rome. Some of the compounds of lead, such as white lead was used as a pottery glaze about 2500 years back, and other lead compounds may have been used as cosmetics from prehistoric period about 5000 years BC. The practice of using galena for eye-paint survives in fact to the present day, particularly in India where it is known as surma, and among Asian immigrants (Winder, 1994)

Although lead was discovered very early in human history, its use and dispersion were greatly increased by developments in silver production, with large spike in lead production at 700-600 B.C. occurring at the advent of coinage. Coinage dates to the time of Lydia (now Turkey) although its widespread adaptation was driven by the Greek islands bordering Lydia (Fitch, 2004), followed by the Persians, then the Egyptians and Romans (Howgego, 1995). Art history data pushes the inception of coinage back to the mid 7th century B.C. (Fitch, 2004). . Lead was particularly used in Egypt in the 2nd and 3d centuries A.D. for small change ((Fitch, 2004), evidence has shown that Egyptian Pharaohs between 3,000 and 4,000 B.C. used lead to glaze pottery. Lead was useful as well in construction. The Babylonians and the Assyrians used soldered lead sheets to fasten bolts and construct buildings. The Chinese used lead to make coins 4,000 years ago, as did the ancient Greeks and Romans. Early warriors made bullets out of it, and gladiators covered their fists with leaden knuckles (Kinder, 1993).

The Romans produced an average of 60,000 tonnes of lead a year for 400 years (Hernberg, 2000)..The ancient Romans used lead for making water pipes and lining baths, and the plumber who joins and mends pipes (Kinder, 1993) They also used to boil and condense grape juice in lead pots for preserving and sweetening of wine(Hernberg,2000). The metal enhanced one-fifth of the 450 recipes in the Roman Apician Cookbook(Kinder, 1993). Lead tokens were frequently used in the Roman era, (Fitch, 2004). Epidemic of lead poisoning from all these sources must have been common in ancient Rome.It has been suggested that widespread lead poisoning, selectively affecting the patricians who drank much wine and had access to plumbing, contributed to the decadence and later the fall of the Roman Empire. Indeed, high lead concentrations have been found in archeological Roman bones; higher in bones retrieved from patrician tombs than in those found in plebeian graves (Hernberg,2000).

Following the fall of Rome in the fourth century, the use of lead declined in Europe and remained at a low level for about 600 years. After the ninth century lead began to be mined in Eastern Germany (Winder, 1994). It is known that white lead was used in England in the thirteenth century as there is reference to its usein 1274(Winder, 1994).. The practice of adulterating wine with lead and its salts had become widespread, and was banned by the 1498. Nevertheless, this continued and epidemics of lead colic were by no means infrequent. Periodic outbreaks of lead colic occurred in 1592, 1793, 1786, 1786 and 1796, however, it was long afterwards before it was discovered by Tronchin to be lead colic (Winder, 1994).

Between 18th and 19th century doctors used lead acetate to treat bleeding and diarrhea, whiskey distilleries used lead tubing to distill alcohol and households frequently used vessels with high lead content to cook and also store drinking water (Geraldine, 2006).

2.5 LEAD SOURCE

Lead occurs naturally in small amount in the earth's crust (Geraldine, 2006), mainly as leaad sulphide (WHO, 2010). Lead in the environment may be derived from either natural or anthropogenic source. Nature source of environmental lead include geological weathering, volcanic emissions. However contribution to human exposure is small. Lead and its compounds entering the environment due to anthropogenic uses such as mining, smelting, processing, recycle and disposal have result in the global disposal of lead with widespread ramifications and far a greater ill effect on human population (Geraldine, 2006).

Recent reductions in the use of lead in petrol (gasoline), paint, plumbing and solder have resulted in substantial reductions in lead levels in the blood (Fewtrell, 2003). However, significant sources of exposure to lead still remain, particularly in developing countries (WHO, 2010). However, the widespread occurrence of lead in the environment is largely the result of human activity, such as mining, smelting, refining and informal recycling of lead; use of leaded petrol (gasoline); production of lead-acid batteries and paints; jewellery making, soldering, ceramics and leaded glass manufacture in informal and home-based industries; electronic waste(WHO, 2010) and use in water pipes and solder. Other sources of lead in the environment include natural activities, such as volcanic activity, geochemical weathering and sea spray emissions, and remobilization of historic sources, such as lead in soil, sediment and water from mining areas. As lead is an element, once it is released into the environment, it persists.Because of lead's persistence and potential for global atmospheric transport, atmospheric emissions affect even the most remote regions of the world (WHO, 2007).

Even though worldwide lead exposure has decreased tremendously in the past decades (Gulson, Mizon *et al.*, 2006), mainly due to the phasing out of leaded gasoline (Mathee *et al.*, 2009), Lead is still ubiquitous. The main source of lead exposure are as follows

2.5.1Air

The transport and distribution of lead from fixed mobile and natural source are primarily via air. (WHO, 2010). Lead is used mainly in the production of lead-acid batteries, plumbing materials and alloys. Other uses are in cable sheathing, paints, glazes and ammunition. Human occupational exposure can also take place during the application and removal of protective lead-containing paints, during the grinding, welding and cutting of materials painted with lead-containing paints, such as in shipbuilding, construction, demolition industries, and fabrication of heavy lead glass and crystal, and in crystal carving. Human extraction and use of lead has (Tong *et al*., 2000) such as mining, smelting, and informal processing and recycling of electric and electronic waste (WHO, 2010) been the main cause of elevated public exposure to environmental lead (Tong *et al*., 2000), and also significant sources of exposure. Lead has been used widely in the form of tetraethyl and tetramethyl lead as anti-knock and lubricating agents in petrol, although the majority of lead is emitted from vehicles in the form of inorganic particles. This use has been phased out in most countries, which has resulted in a significant reduction of human exposure and mean blood lead levels. In the few parts of the world where leaded petrol is still in use, however, it continues to be a major source of exposure. Old industrial hotspots that have not been cleaned up can also represent a hazard (WHO, 2010), even years after contamination has stopped, particularly to children who might ingest contaminated soil or dust as a result of their hand-to-mouth behavior (WHO, 2010).

2.5.2 Food and Smoke

More than 80% of the daily intake of lead is derived from the ingestion of food, dirt and dust (Zheng *et al*., 2008). According to WHO, (2010), for the non-smoking general population, the largest contribution to the daily intake of lead is derived from the ingestion of food, dirt and dust. The amount of lead in food plants depends on soil concentrations and is highest around mines and smelters. Cereals can contain high levels of lead, and spices may be contaminated with lead.

The use of lead-soldered food and beverage cans may considerably increase the lead content of the food or beverage (IPCS, 1995). Milk or formula is a significant source of exposure for infants (WHO, 2000). As alcoholic drinks tend to be acidic, the use of any lead-containing products in their manufacture, distribution or storage will raise lead levels. Migration of lead into food from lead-glazed ceramic or pottery dinnerware is also a source of exposure. Smoking tobacco increases lead intake (IPCS, 1995). Lead also comes to unintentionally contaminate food as the result of contamination with soil or from lead used in machinery to process items , for example, wheels for flour that are coated with lead (WHO, 2000).

Smoke from the open burning of waste may pollute the air and transport lead for long distances, thus reaching communities settled kilometres away from the sources. In some cases, waste may be used as a cheap combustible material to cook or to heat the inside of homes, or around them. Lead is also emitted into the air by incinerators, crematoria, and cement kilns that are old or not well controlled; they pollute the air of entire communities. The WHO air quality guidelines for Europe state that the annual average lead level in air should not exceed 0.5 $\mu g/m^3$(WHO, 2000).

2.5.3 Drinking Water

Lead plumbing has contaminated drinking-water for centuries, and lead in water can contribute to elevated blood lead concentrations in children. In Roman times, water pipes themselves were made of lead. Today the principal source of lead in drinking-water (WHO, 2000). Lead present in tap water is rarely the result of its dissolution from natural sources but is mainly due to household plumbing systems containing lead pipes, solders and fittings. Water that has been in contact with lead in this way for an extended period (e.g. overnight) will have a higher concentration. Thus, lead concentrations can vary over the day, and flushing of the taps before use is a control mechanism. Soft acidic water dissolves the most lead. (WHO, 2008)

2.5.4Domestic Source

Contaminated dust may be the main source of exposure for infants in countries that no longer use leaded petrol. The weathering, peeling or chipping of lead-based paints, mainly found in older houses, plays a role in children’s exposure, especially as some young children eat the fragments or lick dust-laden fingers. Lead-containing dust may be brought into the home on the clothes of those who work in industries where such dust is generated. Some toys either are made from lead or contain lead (e.g. some plastics or paints) (WHO, 2007b). Lead is also present in many household products (WHO, 2000) In some cases, waste may be used as a cheap combustible material to cook or to heat the inside of homes, or around them (WHO, 2000) Children and adolescents of the scavengers and poor families who live close the waste sites may participate actively in these activities to recuperate metals, and sometimes children look for lead to smelt and make sinks to sell.

With the proliferation, and rapid rate of replacement of computers, cellular telephones and other electronic equipment (often lead-containing), the disposal of electronic equipment is emerging as an important lead exposure concern. The concern is greatest in low-income countries because of transfer of electronic waste from high-income countries for disposal (Mathe *et al*, 2009).

Lead is often found in flaking lead-based paints (Mathe*et al*., 2009), candies, cosmetics (Al-Ashban *et al*. 2004), traditional lead-glazed ceramics used in the preparation and serving of food (Mathee *et al*., 2009)

2.6 PATHWAYS OF EXPOSURE

Human exposure to lead may occur mostly through inhalation or ingestion. Ingestion of lead-rich dust or soil. Lead inhalation can also occur during unusual circumstances, such as heat gun stripping of painted surfaces, welding and burning of lead-contaminated items such as batteries, in or near. In these situations, very fine particles of airborne lead are generated and can be inhaled. Young children absorb more lead from the environment than adults (Ahamed and Siddiqui, 2007). Ingestion is the most common route of exposure to lead for children. Once lead has been swallowed, it enters a child's body by absorption from the gastrointestinal tract (EPA, 2002). Inhalation of airborne lead could be a major source of exposure in occupationally exposed adults, because of the particle size of airborne lead in community environments is usually too large to be inhaled by children (Amitai *et al*., 1991). Inhalation can occur, however, when children are exposed to lead in particulate matter less than 10 µm in diameter (PM 10) from car exhausts (in countries that still use leaded gasoline) and smoke from the open burning of waste.(Amitai *et al*., 1991)

Apart from inhalation and ingestion lead exposure occur via dermal absorption. The rate of permeation of lead through the skin will be minimal and depends on the form of lead, with higher rates likely for more soluble forms of inorganic lead (Stauber *et al.*, 1994). A number of publications have shown a statistically significant positive association between dermal lead exposure and blood lead levels (Askin and Volkmann, 1997; Sun *et al.,* 2002). However, as dermal uptake of lead is minimal it is likely that these associations are due to ingestion exposure

after transfer of lead from the hands to the mouth (Cherrie, 2003; Cherrie *et al.,* 2006).

2.7 TOXICOKINETICS OF LEAD

2.7.1 Absorption of Lead

The principal routes of exposure and absorption of lead are through ingestion and inhalation (White *et al*., 1998). Absorption in the gut is partial, influenced by physical form, chemical species of lead, and the presence of other nutrients and dietary cations such as iron and zinc (Hu *et al*., 2006). Gastrointestinal absorption varies depending on nutritional status and age. Iron is believed to impair lead uptake in the gut, while iron deficiency is associated with increased blood lead concentrations in children. (Patrick, 2006a) Calcium supplementation studies demonstrate that increased dietary calcium in animals, infants, and children result in consistent decreases in the absorption of lead(Bogden, 1992). Increased intakes of magnesium, phosphate, alcohol, and dietary fat have also been shown to decrease gastrointestinal absorption of lead(Patrick, 2006a).

GI absorption of lead is greatest in infancy is up to 50 percent of lead ingested from food, water, contaminated dust, or soil, while adults absorb only 10-15 percent.28 Inorganic lead is minimally absorbed through the skin, but tetraethylor alkyl-lead (leaded gasoline), which is still legally allowed in aircraft, watercraft, and farm machinery, is well absorbed through the skin(Papanikolaon e*t al*., 2005)

Inhalation exposure to lead is a much more efficient route of absorption than ingestion, with an estimated absorption efficiency ratio of 10 to 1 in the lung compared to the GI tract (Hodgkin *et* *al*., 1991; Neal and Guilarte, 2012) The deposition of inhaled inorganic lead is dependent on particle size and composition. Larger particles (> 2.5 µm) are deposited in the ciliated regions of the nasopharyngeal and tracheobronchial airways, where they are passed to the gastrointestinal tract by the mucociliary lift mechanism and then subject to intestinal absorption . Particles cleared by mucociliary clearance can be subsequently ingested, contributing to lead exposure via ingestion (ATSDR, 2007). Particles < 1 µm penetrate to alveoli and are subsequently absorbed by phagocytosis(Neal and Guilarte, 2012).

Once absorbed through either ingestion or inhalation, lead enters the bloodstream where it is predominantly bound to erythrocyte proteins (Hu *et al*., 2006) Approximately30-40 percent of inhaled lead is absorbed into the bloodstream (Philip and Gerson, 1994). lead attaches to proteins in the blood that carry it to different tissues or organ systems in the body. Most of the lead present in the blood is bound to the red blood cell. (Gulson and Salome, 1996).

2.7.2 Lead Distribution and Storage

The amount of lead in important organs such as the brain, the hematopoietic system and the kidney are signs of the damage produced by lead accumulation. (Gulson and Salome, 1996)lead enters the bloodstream (Hu *et al*., 2006),in blood, lead is primarily bound to protein. Up to 40% of blood lead is bound to serum albumin, and the remaining blood lead is bound to sulfhydryl- or thiol-containing ligands (Al-Modhefer*et al*., 1991), with an average clearance half-time after a short-term limited exposure of approximately 35 days from whole blood (Rabinowitz, 1991). Clearance occurs through distribution into soft tissues and bone as well as excretion (Hu *et* al., 2006).Lead readily displaces calcium in the bone matrix by cation-exchanges processes (Pounds *et al*., 1991).

Lead cannot be destroyed or changed in the body (Gulson and Salome, 1996).The amount of lead stored in the body has been described as the "body burden" of lead. Among adults over 95% of the total body stores of lead are found in bone. For children about 70% of lead is stored in bone. This lead is not simply stored away in bone forever, but moves in and out as the body functions normally (Gulson and Salome, 1996). Lead circulates widely and is found in all organs and tissues; it also crosses the blood–brain barrier and placenta, making the brain and developing fetus among the targets of concern (Hu, 1998). On a molecular level, lead binds to many proteins, especially to thiol and carboxyl groups, and mimics calcium in many biologic pathways (Rabinowitz 1991).

Work with the radiotracer 203-Pb in rats demonstrated that lead is taken up into the brain most likely as a free ion (PbOH+) or complexed with small molecular weight ligands. PbOH+ most likely crosses the blood brain barrier (BBB) through passive diffusion (Bradbury and Deane, 1993; Yokel, 2006), but could also be transported through cation transporters (Bradbury and Deane, 1993). DMT1 is highly expressed in the striatum, cortex, hippocampus, and cerebellum (Williams *et a*l., 2000) and may facilitate leadtransfer across the BBB (Wang *et al.*, 2011). Brain efflux is likely mediated through ATP-dependent calciumpumps (Yokel, 2006; Marchetti 2003). Within the brain, there is substantial debate regarding lead distribution; some studies have reported that lead preferentially accumulates in specific brain regions, such as the hippocampus (Neal and Guilarte, 2012). However, other studies using different methodologies did not observe any differences in regional brain accumulation of lead(Widzowski and Cory-Slechta , 1994).

2.7.3 Metabolism of Lead

Metabolism of inorganic lead consists of formation of complexes with a variety of protein and nonprotein ligands. Major extracellular ligands include albumen and nonprotein sulfhydryls.

The major intracellular ligand in red blood cells is ALAD. Lead also forms complexes with proteins in the cell nucleus and cytosol. Organic Lead. Alkyl lead compounds are actively metabolized in the liver by oxidative dealkylation catalyzed by cytochrome P-450. Relatively few studies that address the metabolism of alkyl lead compounds in humans have been reported. Occupational monitoring studies of workers who were exposed to tetraethyl lead have shown that tetraethyl lead is excreted in the urine as diethyl lead, ethyl lead, and inorganic lead (Zhang *et al*., 1994; Vural and Duydu 1995). Trialkyl lead metabolites were found in the liver, kidney, and brain following exposure to the tetraalkyl compounds in workers; these metabolites have also been detected in brain tissue of non-occupational subjects (ATSDR, 2007). In volunteers exposed by inhalation to 0.64 and 0.78 mg lead/m3 of 203Pb-labeled tetraethyl and tetramethyl lead, respectively, lead was cleared from the blood within 10 hours, followed by a re-appearance of radioactivity back into the blood after approximately 20 hours (ATSDR, 2007). The high level of radioactivity initially in the plasma indicates the presence of tetraalkyl/trialkyl lead. The subsequent rise in blood radioactivity, however, probably represents water-soluble inorganic lead and trialkyl and dialkyl lead compounds that were formed from the metabolic conversion of the volatile parent compounds (ATSDR, 2007).

2.7.4 Excretion of Lead

Independent of the route of exposure, metabolic balance studies indicate lead is predominately excreted through feces, with urinary excretion playing a secondary role. Trace amounts of lead can also be excreted through hair, sweat, breast milk, and nails(Stauber *et al*., 1994; ATSDR, 2007;Neal and Guilarte, 2012).Fecal excretion accounts for approximately one-third of total excretion of absorbed lead(ATSDR, 2007). The mechanismsfor fecal excretion of absorbed lead are notclearly understood (Patrick, 2006b). However, pathways of excretionmay also include secretion into the bile, gastric fluid, andsaliva, accounting for approximately one-third of totalexcretion of absorbed lead(Patrick, 2006a).

Lead inhaled as submicron particles is deposited primarily in the bronchiolar and alveolar regions of the respiratory tract, from where it is absorbed and excreted primarily in urine and feces(ATSDR, 2007).

Inorganic Lead inhaled as submicron particles is deposited primarily in the bronchiolar and alveolar regions of the respiratory tract, from where it is absorbed and excreted primarily in urine and feces(ATSDR, 2007).Higher fecal-urinary ratios would be expected following inhalation of larger particle sizes (e.g., >1 μm) as these particles would be cleared to the gastrointestinal tract from where a smaller percentage would be absorbed(ATSDR, 2007).

Organic lead inhaledis excreted in exhaled air, urine and feces. Tetramethyl lead gets deposited in the respiratory tract of which approximately 40% gets exhaled in 48hours after inhalation, the lead that is not exhaled gets excreted in urine and feces (ATSDR 2007).Fecal/urinary excretion ratio is about 1.8 following tetraethyl lead exposure and 1.0 following exposure to tetramethyl lead (ATSDR 2007). Occupational monitoring studies of workers who were exposed to tetraethyl lead have shown that tetraethyl lead is excreted in the urine asdiethyl lead, ethyl lead, and inorganic lead (Zhang *et al*., 1994; Vural and Duydu 1995).Inorganic lead is excreted in sweat and urine following dermal exposure to lead nitrate or lead acetate (Stauber *et al*., 1994).

2.8 LEAD HEALTH EFFECT

The health consequences of lead exposure depend on the cumulative dose of lead and vulnerability of the individual person rather than the environmental media (i.e., food, water, soil, dust, or air) in which the lead exists (CDC, 2012). Lead is known to affect virtually every organ or system in the body through mechanism that involves fundamental biochemical processes resulting in a wide variety of changes and substantial risks in living beings (Jacobs, 1996 Simkiss, 2002)Young children are more vulnerable than adults to the effect of lead since they undergo rapid development and their systems are nopt fully developed (Tong *et al*., 2003). The mechanism of toxic effect of lead includes the ability of lead to mimic the action of calcium owing to the possession of the same valency to interact especially with the sulfhyryl group of protein or displacing other essential metal ions. For this reason many organ or organ system have been the potential target of lead in the body, including the hematopoietic, neurological, skeletal, reproductive, gastrointestinal, immune and renal system. (Geraldine, 2006))

2.8.1 Nervous System

Lead can impair cognitive function in children and adults, but children are more vulnerable than adults(WHO, 2000). The increased vulnerability is due in part to the relative importance of exposure pathways (i.e., dust-to-hand-mouth) and differences in toxicokinetics (i.e., absorption of ingested lead) (WHO, 2000). Although the inhalation and oral routes are the main routes of exposure for both adults and children, children are more likely to have contact with contaminated surfaces due to playing on the ground and to hand-to-mouth activities. Furthermore, children absorb a larger fraction of ingested lead than adults (ATSDR, 2007). However, perhaps more important is the fact that the developing central nervous system is especially susceptible to lead toxicity (Su *et al*., 2002). Furthermore, there can be difference in neurological manifestations or sequel between an adult exposed to lead as an adult and an adult exposed as a child when the brain is developing (Canfield *et al*., 2003).Gross toxic effect of lead on the nervous system were first reported by ancient Greek physicianKazantzis, he briefly summarized the description of painters colic. The symptoms of colic which include abdominal pain constipation and paralysis are presently covered by the term “lead encephalopathy” (Geraldine, 2006), which is a general term to describe various diseases that affect brain function (ATSDR, 2007). Lead’s affect on the nervous system in adult includes both central and peripheral nervous systems. Central nervous system involvement in severe lead exposure with blood lead greater than 80µg/dl may cause coma, encephalopathy or death (Geraldine, 2006). The most severe neurological effect of lead in adults is lead encephalopathy, Early symptoms that may develop within weeks of initial exposure include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory, and hallucinations (ATSDR, 2007). The condition may then worsen, sometimes abruptly, to delirium, convulsions, paralysis, coma, and death (ATSDR, 2007). Histopathological findings in fatal cases of lead encephalopathy in adults are similar to those in children (ATSDR, 2007). Lead may also damage the BBB and subsequently brain tissues (Geraldine, 2006). Symptoms from individuals ranging from lethargy to convulsion following consumption of illitictly distilled whisky (Geraldine, 2006). Symptoms typically which occur with low to moderate exposure in lead exposed workers include malaise, forgetfulness, irritability, headache, tiredness, despression, increased nervousness, dizziness and anxiety(ATSDR, 2000). The toxic manifestations of lead in the peripheral nervous system include neuropathy also called lead palsy (Marsden, 2003). Lead based industrial workers as well as individuals with long term lead exposure often present with weakness of the forearm extensor nerve leading to wrist drop, a classic symptom of peripheral neuropathy appearing at higher blood lead levels around 60µg/dl or greater. However, the effect is reversible when the exposure ceases, the recovery begins slow (Barats *et al.*, 2000). Slowed nerve conduction is also a classic symptom on workers chronically exposed to high lead level (Geraldine, 2006) However, Gidlow, (2004) stated in a body of literatureclinical significance of reduced nerve conduction velocity is uncertain.Studies have shown a slowing of sensory motor reaction time in male lead workers and some disturbance of cognitive function in workers with blood lead levels >40 μg/100 ml. Peripheral motor neuropathy is seen as a result of chronic high-level lead exposure, but there is conflicting, although on the whole convincing, evidence of a reduction in peripheral nerve conduction velocity at lower blood lead levels. The threshold has been suggested to be as low as 30 μg/100 ml, although other studies have not seen effects below a blood lead level of 70 μg/100 ml (Davis and Svensgaard, 1990)

Subtle changes in neuropsychological function have been seen in inorganic lead workers. These effects are seen in visual/motor performance, memory, attention and verbal comprehension (Hannimen, 1998; Gidlow, 2004). These effects can be detected in workers with blood lead levels of >50 μg/100 ml, but it is claimed that sensory motor function is more sensitive than cognitive function and effects may be observed at blood lead levels as low as40 μg/100 ml (Gidlow, 2004). Many of these tests have been wellperformed and used non-exposed controls who had been well-matched for educational achievement. However, there are other variables that have not been adequately controlled, e.g. alcohol consumption or the incidence of hypertension and cerebrovascular disease. One interesting study has shown a subjective improvement in levels of tension, anger, depression, fatigue and confusion following a significant improvement in occupational exposure and reduction in blood lead levels, but no significant improvement in the subtle neuropsychologicaltest results (Gidlow, 2004).

Much debate surrounds the potential effects of low-level lead exposure on young children. There is no doubt that subtle effects on child neuropsychological development can be seen at blood lead levels above 20 μg/100 ml. Moreover, one study has produced data suggesting effects below

10 μg/100 ml with no discernible no-effect level (Cranfeild, 2003).During brain development, lead interferes with the trimming and pruning of synapses, migration of neurons, and neuron/glia interactions. Alterations of any of these processes may result in failure to establish appropriate connections between structures and eventually in permanently altered functions (ATSDR, 2007. Because different brain areas mature at different times, the final outcome of the exposure to lead during development (i.e., *in utero* vs. pediatric exposure) will vary depending on the time of exposure. This has been demonstrated in studies in animals (ATSDR, 2007). The time of exposure-specific response appears to have contributed to the failure to identify a "behavioral signature" of lead exposure in children. Other factors that may affect individual vulnerability are certain genetic polymorphisms, such as that for the vitamin D receptor, the lead-binding enzyme ALAD, or the APOE genotype (ATSDR, 2007).

Lead in children appears to interrupt and inhibit the neural differentiation, pathway development and learning ability, thus causing a life long decrement in intelligence. There is a large body of evidence that associates decrement in intelligence quotient (IQ) performanceand other neuropsychological defects in children with lead exposure. Canfeild *et al*.(2003) evaluated the neurobehavioral functioning of children having blood lead greater than 10µg/dl, a CDC level of concern, and measured blood lead level of different children and tested IQ scores at three and five years of age. The result indicated that blood lead level about 10µg/dl were inversely associated with children's IQ scores, Chiodo *et al*.(2004) examine a specific neurobehavioral deficit in relation to low blood lead level in areas of intelligence, reaction time, visual-motor integration, fine motor skills, attention (including executive function), off-task behaviour and teacher report behaviour and found association in blood lead level as low s 3µg/dl. Further research provided additional support for the conclusion that neurobehavioral deficit terms are constantly associated with blood lead levels below 10µg/dl and suggested that it may be apprioprate for CDC and EPA to re-evaluate the 10µg/dl to concern correctly articulated for post natal lead exposure (Chiodo, 2004)

Acute exposure in children to very high lead may produce lead encephalopathy and its attendant signs like convulsion, stupor,hyperirritability, coma, etc. In this context, Herman *et al*., (2002) reported lead encephalopathy in a five months old born to a mother with secondary occupational exposure. A casual relationship between lead exposure and aggressive behaviour in lead exposed young children was shown by Li *et al*., (2003) using feline model by measuring the predatory attack threshold current. Lead exposure enhances the predatory aggression in cats and provided the experimental support for a casual relationship between lead exposure and aggressive behaviour in human.

2.8.2 Reproductive System

A number of studies have examined the potential association between lead exposure and reproductive parameters in humans (ATSDR, 2007). The available evidence suggest that occupational and environmental exposure resulting in moderately high blood lead levels might result in abortion and pre-term delivery in women, and in alterations in sperm and decreased fertility in men (ATSDR, 2007). Lead intoxication in female is associated with delayed sexual maturituration, sterility, miscarriage, stillbirth and effects upon the foetus, premature rapture of membrane, pre-term delivery and decreased birth weight of newborn. (Gonzalez-Cossfo *et at*., 1994). At very high blood lead levels, lead is a powerful abortifacient. At lower levels, it has been associated with miscarriages and low birth weights of infants(Gidlow, 2004). Predominantly to protect the developing fetus, legislation for lead workers often includes lower exposure criteria for women of 'reproductive capacity' (Gidlow, 2004). ATSDR (2007) reported that female workers at a lead smelter in Sweden had an increased frequency of spontaneous miscarriage when employed during pregnancy (294 pregnancies, 13.9% ended in spontaneous abortion) or when employed at the smelter prior to pregnancy and still living within 10 km of the smelter (176 pregnancies, 17% ended in spontaneous abortion). The abortion rates in these two groups of pregnant women were significantly higher than in women who were pregnant before they became employed at the smelter and in women who became pregnant after employment but lived more than 10 km from the smelter. Although no environmental or biological monitoring for lead was available, women who worked in more highly contaminated areas of the smelter were more likely to have aborted than were other women. A nested control-case study of a cohort of 668 pregnant women in Mexico City showed that the risk of spontaneous abortion (defined as loss of pregnancy by gestation week 20) increased with increasing blood lead (Borja-Aburto *et al*., 1999). Some studies demonstrated a reduction in dam weight gain during gestation and reduction in average pup weight born to rats receiving lead (Geraldine, 2006). Other studies reported prenatal and postnatal exposure to lead alters the affinity and number of estradiol receptors in the prepubertal and adult rats uterus (Geraldine, 2006). Daarthi *et a*l., (2002)found delayed puberty associated with suppressed serum level an insulin-like growth factor-1 (IGF-1), leuteinizing hormone, and estradiol in rats exposed to lead prenatally. Gorbel *et al*., (2002) In his study showed double sexual disorder in rats, firstly, with hormonal function during initial stage of exposure and secondly, disorder of the genital tract affecting the ovary resulting in reduced fertility in spite of the presence of normal oestrus. However, as concerns reproduction, the adverse action of lead is reversible after withdrawal of the female from lead source (Piasek and Kostial, 1991).

The reproductive toxicity of lead on male lead workers has been studied but, to date, the results have been inconsistent (Lerada, 1992; Telisman *et al.*, 2000).Lead toxicity has been associated with decreased libido, premature ejaculation, erectile dysfunction and reduced semen volume (Marsden, 2003). High levels of lead exposure may result in decreased sperm count and mobility, an increased number of morphological abnormal sperm,decreased weight of testes and epididyes, seminal vescicle and prostate (Marchlewicz *et al.*, 1993; Pinon-Latailland *et al.*, 1995). Some studies showed high semen lead and significantly shifted distribution of sperm count in subjects exposed to high lead levels (Geraldine, 2006) Other studies studies show testicular damage in rats following oral lead administration (Batra *et al.*, 1997).Some workers occupationally exposed to inorganic lead showed a significant reduction in fertility relative to unexposed men (Sállmen *et al.*, 2000). The risk ratio (RR) for infertility in exposed men appeared to increase with increasing blood lead level; thus, the RRs for the blood lead level categories 10–20, 21–30, 31–40, 41–50, and ≥51 μg/dL were 1.27, 1.35, 1.37, 1.50, and 1.90, respectively; however, there was no evidence of decreased fertility in couples who had achieved at least one pregnancy (ATSDR, 2007). A study of 163 Taiwanese male lead battery workers showed decreased fertility in men with PbB in the range of 30–39 and ≥40 μg/dL, but there was no significant reduction in fertility in men with blood lead level of ≤29 μg/dL (Shiau *et al.*,2004). Workers with the highest cumulative exposure to lead had the most marked reduction in fertility (ATSDR, 2007).

A study of 163 Taiwanese male lead battery workers showed decreased fertility in men with blood lead level in the range of 30–39 and ≥40 μg/dL, but there was no significant reduction in fertility in men with PbB of ≤29 μg/dL (Shiau *et al.*, 2004). There was no effect on fertility among men (n=229) employed in a French battery factory (Coste *et al.*, 1991) or among Danish men (n=1,349) exposed to lead (mean blood lead level of a subset of 400 workers, 39.2 µg/dL) during the manufacture of batteries (Bonde and Kolstad, 1997). There was weak evidence of increased time-to-pregnancy (TTP) in the wives of 251 occupationally-exposed men in Finland with blood lead level ranging from 10 to 40 µg/dL or higher (Sállmen *et al.* , 2000). The study included only couples who had at least one pregnancy and the association was limited to men whose wives were less than 30 years old. A study with similar exposure levels in 251 Italian men did not find an association between lead exposure in men and delayed TTP in their wives (ATSDR, 2007). There was no association between occupational exposure to lead and low fertility in a multi-country study of 638 men exposed occupationally to lead (Joffe *et al.*, 2003). Mean blood lead level in exposed men ranged from 29.3 to 37.5 µg/dL, but most was below 50 µg/dL. Although the evidence for reduced fertility is not conclusive, it appears that a threshold for fertility effects in men could be in the blood lead level range of 30–40 µg/dL.Some studies have shown reducedsperm count and motility, but there are few data showingan effect on reproductive capability(Gidlow, 2004). In addition, many of the studies havenot taken into account potentiallypowerful confounding factors such as other occupationalexposures (e.g. heat and solvents) or social factors suchas alcohol consumption, smoking or the use of anymedications (Gidlow, 2004).

A study of 503 lead workers in theUK, Belgium and Italy examined semen samplesaccording to an agreed protocol. The results showed a 49% reduction in the median sperm concentration inmen with blood lead levels >50 µg/100 ml, with a likelythreshold level for effects of 44 µg/100 ml. In addition,there was some evidence of deterioration of spermchromatin in men with the highest concentration of leadin spermatozoa. Biological monitoring data failed to showany long-term effects of lead on sperm quantity or spermchromatin (Bonde *et al.*, 2002). However

ATSDR, (2007) reported that although there is some variation in the results, most of the available studies suggest that reductions in sperm concentration, indications of adverse effects on sperm chromatin, and evidence of sperm abnormalities may occur in men with mean blood lead levels> 40 µg/dL but not in men with lower blood lead levels. A study of 81 lead smelter workers showed an association between blood lead level and sperm concentration (ATSDR, 2007). In addition, although blood lead level concentrations were not related to serum testosterone, a reduction in serum testosterone with increasing semen lead concentration was observed (ATSDR, 2007).

A cross-sectional study of 149 industrial workers in Zagreb, Croatia, found that 98 men who had moderate occupational exposure to lead (mean blood lead level, 36.7 µg/dL) had significantly lower sperm density, and lower counts of total motile and viable sperm; lower percentage and count of progressively motile sperm; higher prevalence of morphologically abnormal sperm head; and lower level of indicators of prostate secretory function compared with 51 referents (mean PbB, 10.3 µg/dL) (Telisman *et al.*, 2000).No significant differences were found for semen volume or percentages of motile, viable, and pathologic sperm. Workers also had significantly higher serum estradiol than the refernce group, but there were differences in serum FSH, LH, prolactin, and testosterone levels (Telisman *et al.*, 2000).

Current thinking is that significant effectson reproductive capacity are not seen below a blood leadlevel of ≥50 µg/100 ml, but blood lead concentrations of>40 µg/100 ml may affect sperm morphology andfunction (Apostoli *et al.*, 1998).

A low dose of lead during early development of male reproductive system alters the composition of protein and nucleic acids, interfering with normal spermatogenesis process. Apart from the testes, the seminal vesicles and prostate glands are also targets of lead toxicity (Corpas *et al*., 2000).

2.8.3 Developmental Effect

No reports were found indicating low levels of lead as a cause of major congenital anomalies. However, in a study of 5,183 consecutive deliveries of at least 20 weeks of gestation, cord blood lead was associated with the incidence of minor anomalies (hemangiomas and lymphangiomas, hydrocele, skin anomalies, undescended testicles), but not with multiple or major malformations (ATSDR, 2007). In addition, no particular type of malformation was associated with lead. According to the investigations, the results suggested that lead may interact with other teratogenic risk factors to enhance the probability of abnormal outcome (ATSDR, 2007).

Two studies provide information on the effect of lead exposure on sexual maturation in girls. Selevan *et al*., (2003) performed an analysis of data on blood lead concentrations and various indices of sexual maturation in a group of 2,741 female children and adolescents, ages 8–18 years,. They observed that increasing blood lead was significantly associated with decreasing stature (height) and delayed sexual development (lower Tanner stage, a numerical categorization of female sexual maturity), after adjusting for covariates. Covariates included in the models were age, height, body mass index; history of tobacco smoking or anemia; dietary intakes of iron, vitamin C and calcium; and family income. Selevan *et al*., (2003) acknowledged that other factors associated with body lead burden and pubertal development that they did not assess may be responsible for the observed associations. In addition, they noted that reporting of past events, such as age at menarche and dietary history could have been subject to errors in recall. Finally, potential confounders that were measured at the time of the study may have differed during periods critical for pubertal development or other unmeasured confounders may have affected the results.

An additional study of the same cohort also found a significant and negative association between blood lead level and delayed sexual maturation (Wu *et al*., 2003). The study included 1,706 girls 8–16 years old with blood lead level ranging from 0.7 to 21.7 μg/dL. Blood lead levels were categorized in three levels: 0.7–2, 2.1–4.9, and 5.0– 21.7 μg/dL. Covariates included in the models were race/ethnicity, age, family size, residence in a metropolitan area, poverty income ratio, and body mass index. Girls who had not reached menarche had higher blood lead level than did girls who had. Among girls in the three levels of blood lead level mentioned above, the unweighted percentages of 10-year-old girls who had attained Tanner stage 2 pubic hair were 60, 51, and 44%, respectively, and for 12-year-old girls who reported reaching menarche, the values were 68, 44, and 39%, respectively. These negative relationships remained significant in logistic regression even after adjustment for the covariates mentioned above. Interestingly, no significant association was found between blood lead levelsome development, in contrast to the findings of Selevan *et al*., (2003) who used the same database. Wu *et al*., (2003) concluded that although they found a significant negative association between low blood lead level and some markers of sexual maturation, judicious interpretation of the results is needed given the cross-sectional study sample and limited attention to other nutritional or genetic factors that may impact the findings.

Some studies have reported delays in sexual maturation in animals exposed to lead, although associated with blood lead levels much higher than those measured in girls in the Selevan *et al*., (2003) and Wu *et al*., (2003) studies(ATSDR,2007).In a stdy conducted by Ronis *et al*., (1996) exposure of male and female Sprague-Dawley rats prepubertally (age 24–74 days) to lead acetate in the drinking water resulted in significant reduction in testis weight and in the weight of secondary sex organs in males and in delayed vaginal opening and disruption of estrus cycle in females. However, these effects were not observed in rats exposed postpubertally (day 60– 74 in males, 60–85 in females).

Increasing evidence indicates lead readily crosses the placenta and causes developmental defects, which includes pregnancy outcomes such as pre-term delivery, featal neurotoxicity, intrauterine growth restriction and post birth effects on growth or neurological development (ATSDR, 2000; Atonio and Leret, 2000).Some parameters in animal model in discrete brain areas of the pups whose mothers were exposed to low levels of lead via drinking water aduring pregnancy and lactation, a significant reduction in the activity of enzymes alkaline phosphatise and ATPase in the brain were found. There was general decrease in neurotransmitter levels in all areas especially the hippocampus which could be related to the decrease in the synthetic capacity, thus explaining the neurophysiologicala and neurobehavioral changes in lead intoxicated animals (Trope *et al.*, 2000)

Following a study of the animal model hypothesized that the lead induced associative deficit may reflect lasting damage to the amygdale and perhaps nucleus accumbens (Garavan *et al.*,1999)

2.8.4Skeletal System

Approximately 90-95% of lead is stored in calcium-dependant pools, with turnover especially in the cortical bone.(Marsden, 2003) Bone is the highest depository of the body burden of lead. The human skeleton begins to accumulate lead during featal development and continue throughout much of human span. However, cessation of lead exposure can lead to decrease in bone lead.

(Geraldine, 2006). Lead may directly or indirectly alter several aspects of bone cell function by changing the circulation level of the hormone1,25-dihydroxyvitamin D, inhibiting the synthesis of osteocalcin and impairing the ability of cells to synthesize or secrete collagen. Lead may directly substitute for calcium in the active site for calcium messanger resulting in loss of physiological regulations (Pound, 1991).Skeletal lead is not metabolically inert, but may be mobilized during a number of physiological and pathological conditions involving increased bone turnover as age, endocrine atatus, osteoporosis, menopause, renal diseases and in particular during pregnancy and lactation for deman of calcium, thus acting as an endogenous source of lead. (Berkowitz, 1999) Human bone appears to have at least kinetically distinct lead compartments lead in trabecular (spongy) bone appears to be moremobile than lead lodged in cortical (compact) bone. Thus it becomes quite conceivable that the mobilization of lead from bone to the more bioavailable meternal blood compartment poses a risk to the fetus and mother (Geraldine, 2006).

2.8.5 Renal System

Lead nephropathy has been well documented in occupationally exposed workers. It manifests asproximal tubular damage, glomerular sclerosis, and interstitial fibrosis(Patrick, 2006a). Signs include proteinuria, impair.redtransport of glucose and organic anions, andlowered glomerular filtration rate (GFR)Classically, renal insufficiency is found in acute leadtoxicity and is accompanied by abdominal pain (leadcolic), cognitive defects, peripheral neuropathy, arthralgias,anemia with basophilic stippling, a “leadline” at the junction of the teeth and gums, and highlead blood levels >80 μg/dL(Marsden, 2003). However, there is significant evidencethat renal damage occurs at much lower exposurelevels in the general population. Multiple studiesdefine a strong relationship between blood lead levelsand a decline in renal function associated with age instudy populations not occupationally exposed(Staessen *et al*., 1992; Payton *et* al., 1994) In these studies, significant correlations exist betweenblood lead levels <10μg/dL and elevations in serum creatinine;serum creatinine increased 0.14mg/dL for every 10-fold increase in blood lead. These studies haveadmitted limitations because they did not excludeconfounding factors, including hypertension, use ofangiotensin-converting enzyme inhibitors, or urinaryprotein excretion(Patrick, 2006a). A prospective trial of 448 adults, a subsampleof the Normative Aging Study, found low-level leadaccumulation wasassociated with an increased risk for declining renalfunction (measured by increased serum creatinine)(Tsaih *et* al., 2004). This was more pronounced in diabetics and hypertensives,who are already at risk for renal disease. The mean baseline and six-yearfollow-up lead blood levels in this population would otherwisebe considered normal – 6.5μg/dL and 4.5μg/dL, respectively(Patrick, 2006a)

However, in the diabetic subpopulation, both blood and bone lead levels were associated withsignificant increases in serum creatinine. When comparedto non-diabetics, those in the highest quartile ofbone lead had 17.6-fold higher serum creatinine andthose in the highest quartile of blood lead had 12.8-fold higher serum creatinine. In a prospective trial, EDTA-mobilizationtests demonstrated that chronic low-level lead exposureis related to the progression of chronic renalinsufficiency(Patrick, 2006a)The trial revealed a significant relationship between blood lead levels, body burdenas diagnosed by EDTA mobilization, and GFR. Anelevatedbody lead burden was defined as 600μgurine lead in a 72-hour collection after infusion of 1 gcalcium disodium EDTA. Of 121 patients with chronicrenal insufficiency, body lead burden and bloodlead levels weresignificant predictors of progressionof renal disease, the body lead burden being the mostpowerful predictor, followed by male genderand the presence of chronic interstitial nephritis. Seventeen patients progressed (defined by a doubling of serumcreatinine), and 15 of those had what were considered high normal body lead burdens – 80-600µg and normal lead blood levels (4.9 ± 2.6µg/dL)(Patrick, 2006a).

2.8.6 Immune System

Numerous studies have examined the effects of lead exposure on immunological parameters in lead workers and a smaller number of studies provide information on effects in members of the general population, including children. The results although mixed, give some indication that lead may have an effect on the cellular component of the immune system, while the humoral component is relatively unaffected. However, the clinical significance of these relationships is as yet unknown (ATSDR. 2007).

Lead appears to reduce the resistance and increase themortality of experimental animals (Koller, 1985). It apparentlyimpairs antibody production and decreases immunoglobulinplaque-forming cells. There is some evidence forsuggesting that workers with blood lead levels between20 and 85µg/100 ml may have an increased susceptibilityto colds , but a study of lead workers with bloodlead levels of <50 µg/100 ml showed no significantimmunological changes (Gidlow, 2004). An increased percentageand increased absolute count of B lymphocytes may beseen in workers with blood lead levels of >50 µg/100 ml(Gidlow, 2004).

2.8.7 Cardiovascular System

Lead has been shown to produce various cardiovascular effects in animals (Vaziri and Sica 2004). Elevated blood lead levels (20-29 µg/dL) correlate withsignificant increases in all-cause circulatory and cardiovascularmortality. (Lustberg and Sibergeld, 2002) Several clinical trials andpopulation studies of occupationally exposed groupshave shown that lead exposure correlates with increasedincidence of hypertension, cerebrovasculardisease, and cardiovascular disease(Sakos *et* al., 1997; Patrick, 2006a). There is substantialevidence that long-term, low-level exposureto lead can contribute to hypertension in both animalsand humans (ATSDR, 2005). The data on hypertension and itsrelationship to lead exposure is well established inthe medical literature. (Patrick, 2006a) Three meta-analyses of 61 collectivestudies show a positive relationship betweenincreasing blood lead levels and elevated systolic anddiastolic blood pressure.(Staessen *et al*., 1994; Schwarts, 1995; Newrot *et al*., 2002) Although these metaanalysesreveal only small rises in arterial pressures(1.0-1.25 mmHg and 0.6 mmHg for every doublingof blood lead levels (Mulrow, 1998).The increased risk of elevated blood lead inpostmenopausal women secondary to bone resorptionyields a concomitant risk for hypertension (Nash*et al*., 2003). Inan analysis of 2,165 pre- and postmenopausal women,blood lead levels and incidence of hypertensionwere analyzed (Nash*et al*., 2003). Among women ages 40-59 years,both pre- and postmenopausal, those in the highestquartile of blood lead levels, with mean value 6.3 µg/dL, had a 3.4-fold increased risk for diastolic hypertension, comparedwith the lowest quartile of blood lead levels, mean value1.0 µg/dL(Nash*et al*., 2003). Looking at only postmenopausal women,those in the highest quartile of blood lead levels had an 8.1-foldincreased risk for diastolic hypertension(Nash*et al*., 2003). Large scaletrials have shown that postmenopausal women are atincreased risk for hypertension, and that loss of estrogenis directly associated with this risk (Staessen *et al*., 1998) There areas yet no statistical analyses of the effects of loweredestrogen on bone resorption of lead into the systemiccirculation(Patrick, 2006a). Navs-Acien*et al*.,(2004) reported in an analysis of 2,125 participants in the NHANES 1999-2000, blood lead and cadmium levels were correlated with the incidence of peripheral arterial disease (PAD) based on ankle-brachial blood pressure indices.For the large majority (98.3%) of subjects in the study, blood lead levels measured under 10 µg/ dL, even after adjusting for GFR and C-reactive protein, ruling out inflammation and impaired renal function (Patrick, 2006a). Tibia lead levels were found to be significantly associated with electrocardiographic changesin men ages 48-93. (Cheng *et al.*, 1998) Those under 65 had a significantincrease in QT and QRS intervals for every 10-µg/gincrease in tibia lead. After adjusting for high densitylipoprotein (HDL) and age, men under65 with the same incremental increase in tibia leadhad a 2.23-fold greater risk for intraventricular block(Patrick, 2006a).In men over 65 years, each 10-µg/g increase in tibiallead resulted in 1.2-fold higher risk for atrioventricularblock. Blood lead levels in these men (5.8 µg/dL ±3.4) were not associated with tibia lead levels.(Patrick, 2006a)

2.8.8 Carcinogenic Effect

Based on limited evidence from studies in humans and sufficient evidence from animal studies, the U.S. Department of Health and Human Services (HHS) has determined that lead and lead compounds are reasonably anticipated to be human carcinogens, and the U.S. Environmental Protection Agency (EPA) has determined that lead is a probable human carcinogen. The International Agency for Research on Cancer also has determined that inorganic lead is likely carcinogenic in humans (ATSDR, 2007).Lead exposure has been associated with increased risk of lung, stomach, and urinary-bladder cancer in diverse human populations (Fu and Boffetta 1995, Steenland and Boffetta 2000, NTP 2003). The strongest epidemiological evidence is for lung and stomach cancer, which are consistently but weakly associated with occupations and industries entailing lead exposure and with indices of individual lead exposure, including job history and biological monitoring of occupationally exposed and general populations(NTP, 2011). However, most studies of lead exposure and cancer reviewed had limitations, including poor exposure assessment and failure to control for confounding by other factors that could increase the risk of cancer, such as lifestyle factors and concurrent occupational exposure to other carcinogens, and did not demonstrate relationships between the level or duration of exposure and the magnitude of cancer risk. The crude exposure measures used in most studies, such as treating whole plants or occupations as having uniform exposure, may have limited the magnitude of risk estimates, most of which were modest(NTP, 2011). Evidence from epidemiological studies therefore is compatible with small increases in the risk of lung or stomach cancer; however, this evidence must be weighed against the potential for confounding by factors such as smoking, diet, or co exposure to arsenic(NTP, 2011).

2.8.9 Effect on Other Organs

Thyroid Gland

Lead exposure in animal experiments and cases of severe lead poisoning has been reported to show reduction of the functioning of the thyroid gland. There has been a few reports on lead affecting the thyroid function at high blood lead levels. Lin and Shih, (1997) indicated lead as a major aetiological factor inducing the hypothyroid state in a patient with elevated lead burden and chronic renal insuffiecency. They hypothezed that lead may play an important role in thyroxin synthesis or alternatively lead may be a non-specific toxin contributing to the hypothyroid state in patients with pre-existing renal insufficiency(Lin and Shih, 1997). However, the effect of low levels of environmental lead exposure on the thyroid function of persons without previous lead exposure remain unclear (Geraldine, 2006).

The Lungs

There seems to be limited information on the effect of lead on animals and human beings. Nevertheless, morphological alterations have been reported in lungs of lead intoxicated rats. High concentration of lead with prolong exposure on the rat'slungs shows mononuclear cell invasion in the interalveolar septa and collagen fiber accumulation characterized by fibrosis and bronchopneumonia characterized by lymphocyte accumulation in the alveolar and bronchiolar (Onarlioglu *et al*., 1999).

Tooth

The role of lead in the etiology of dental caries has been evidenced.(Geraldine, 2006), as cited by Bowen, (2001) observed a lead line in the continuously erupting incisor of rats following a single injection of a large dose of lead acetate. Further examination of this line revealed irregular tubular structures and uneven mineralization probably because of incomplete fusion of small calcospherites. These observation suggested that lead may affect odontoblast function and thereby influence dentine formation.

2.9 LEAD AND THE HEMATOPOIETIC SYSTEM

The hematopoietic system is one of the major target of lead. One important mechanism of lead is its effect on several enzymatic reactions critical to heme biosynthesis (Geraldine, 2006) The key enzyme involved in the synthesis of heme are δ-aminolevulinic synthetase (ALAS),a mitochondrial enzyme that catalyse the formation of δ-aminolevulinic synthetase (ALA), and δ-aminolevulinic acid dehydratase (ALAD), a cytosolic enzyme that catalyse formation of porphobilinogen (Geraldine, 2006). Over 90% of lead present in the blood accumulates in erythrocytes greater than 80% of lead in erythrocytes binds to ALAD and cause its inhibition (Onailaja and Claudeo, 2000). The anemia induced by lead is microcytic and hypochromic and results primarily from both inhibition of heme synthesis and shortening of the erythrocyte lifespan. Lead interferes with heme synthesis by altering the activities of δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase. As a consequence of these changes, heme biosynthesis is decreased and the activity of the rate-limiting enzyme of the pathway, δ-aminolevulinic synthetase (ALAS), which is feedback inhibited by heme, is subsequently increased. The end results of these changes in enzyme activities are increased urinary porphyrins, coproporphyrin, and δ-aminolevulinic acid dehydratase (ALAD); increased blood and plasma ALA; and increased erythrocyte protoporphyrin (EP) (ATSDR, 2007).

Studies of lead workers have shown that ALAD activity correlated inversely with blood lead (Schuhmacher *et al.*, 1997; Gurer-Orhan *et al.*, 2004), as has been seen in subjects with no occupational exposure (ATSDR, 2007). Erythrocyte ALAD and hepatic ALAD activities were correlated directly with each other and correlated inversely with blood lead in the range of 12–56μg/dL (ATSDR,2007). General population studies indicate that the activity of ALAD is inhibited at very low blood lead, with no threshold yet apparent. ALAD activity was inversely correlated with blood lead over the entire range of 3– 34 μg/dL in urban subjects never exposed occupationally (ATSDR, 2007). Studies of children in India and China also have reported significant decreases in ALAD activity associated with PbB ≥10 μg/dL (Ahamed *et al.*, 2005; Jin *et al.*, 2006). Inverse correlations between blood lead level and ALAD activity were found in mothers (at delivery) and their newborns (cord blood). Blood lead level ranged from approximately 3 to 30 μg/dL (ATSDR, 2007).

ALAD has 2 isoforms ALAD2 and ALAD1 as a result of polymorphism of the ALAD gene. ALAD2 appears to have higher affinity for lead than does ALAD1. Some studies have shown evidence that ALAD2 keeps the lead csequested in the blood, the increased affinity of the ALAD2 phenotype decreases the entry of lead from the blood into the tissue (Bellingor *et al.*, 1994). However. The substitution of aspargine for lysine at the residue 59 resulting from a single nucleotride change the position177 of coding region, appears to change the electrical charge of molecule resulting in ALAD2 having higher affinity for lead than ALAD1 (Olaiz, 1996). Despite the potential diagnostic utility of ALAD measurements in lead poisoning, the instability of tha enzyme demands its analysis within 24 hours of blood sampling and thus limits its practical utility for surveilliance. Moreover, the inhibition of the enzyme is so extensivge, at blood lead levels of 30µg/dl, that the assay can not readily distinguish between moderate and severe exposure (Grazaino, 1994). Despite the fact that ALAU is relatively insensitive measure of lead toxicity owing to its excretion at high lead blood level, it has found importance in screening lead exposured workers with chronic lead exposure and in parts of the world where environmental lead exposure is extensive and financial resources limited. However, it is non invasive and simple to measure (Grazaino, 1994).Lead also interferes with functioning of intramitichondrial ferrochelatase, which is responsible for insertion of iron (11) into the protoporphyrin ring to form heme. The inhibition of the enzyme leads to binding of zinc to the protoporphyrin resulting in zinc protoporohyrin with a protein remaining free. This decreases the synthesis of heme causing anaemia (Geraldine, 2006).

2.10 OXIDATIVE STRESS

Oxidation provides energy for maintenance of cellular integrity and functions. Most of the consumed oxygen forms carbon dioxide and water; however, 1 to 2% of the oxygen is not completely reduced and forms reactive oxygen species (Clarkson and Thompson, 2000). Oxidative stress, a pervasive condition of increased amounts of reactive oxygen species, is now recognized to be a prominent feature of many acute and chronic diseases and even of the normal aging process (Dalle-Donne, 2006).Oxidative stress occurs when generation of free radicals exceded the capacity of antioxidant defense mechanisms (Sharma *et at*., 2011).

Increased oxidative stress generally describes a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species generated because of excessive production of reactive oxygen species loss of antioxidant defenses, or both (William *et al.*, 2004). Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione (Gems, 2008). Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage(Valko *et al.*, 2006). When antioxidant systems are insufficient, oxidative processes may damage DNA and contribute to degenerative changes, including aging (William *et al.*, 2004). DNA damage can be induced by ionizing radiation which is similar to oxidative stress, and these lesions have been implicated in aging and cancer (Leli *et al.*, 1998). In humans, however, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens (Segal, 2005). Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis (Lennon *et al.*, 1991).

The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon, 1999). A major consequence of oxidative stress is damage to nucleic acid bases, lipids, and proteins, which can severely compromise cell health and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis. Oxidative damage of any of these biomolecules, increasing amount of evidence suggests that oxidative stress is linked to either the primary or secondary pathophysiologic mechanisms of multiple acute and chronic human diseases. However, definitive evidence for this association has often been lacking because of recognized shortcomings with methods available to assess oxidative stress status in vivo in humans (Haliwell and Whiteman, 2007). Substantial evidence suggests that reactive oxygen species participate in the normal aging process as well as in age-related diseases such as atherosclerosis and ophthalmologic and neurodegenerative diseases (Finkel and Holbrook, 2000; Finkel 2003) such as Parkinson's disease, Alzheimer's disease (Valko *et al*, 2007), oxidative stress is also thought to be involved in the development of atherosclerosis, heart failure, (Singh *et a,l* 1995) myocardial infarction (Ramond *et al*, 2011; Dean *et at*, 2011) fragile X syndrome (deDiego-Oterp, 2009), Sickle Cell Disease (Amer, 2006), lichen planus (Aly, 2010), vitiligo (Arican and Kuturas, 2008), autism (James *et al*, 2004), and chronic fatigue syndrome (Kenndy *et al*, 2005). Furthermore, observations in vitro and in cultured cell systems indicate that oxidative stress contributes to cancer risk by numerous mechanisms that are independent of genotoxicity (Dedon, 2004). Recent evidence has further supported the association between the cellular response to oxidants and the mechanisms that regulate longevity.

It is now well established that biological aging correlates with the accumulation of oxidized biomolecules in most tissues (Stadtman and Barlett, 1997; Linton *et al*, 2001; Schoneichie, 2005). In the study of age-related increases in concentrations of oxidized biomolecules, disparities have been observed between intracellular and extracellular proteins. The concentrations of oxidative markers were found to increase more with age in extracellular proteins than in intracellular proteins (Linton *et al*., 2001). This disparity might be explained by a difference in turnover between extracellular and intracellular proteins. The difference in homeostatic control between extra- and intracellular proteins might also play a role (Dalle-Donne, 2006).

The localization and effects of oxidative stress, as well as information regarding the nature of the reactive oxygen species may be gleaned from the analysis of discrete biomarkers of oxidative stress/damage isolated from tissues and biological fluids. Several in vitro markers of oxidative stress are available, including reactive oxygen species themselves, but most are of limited value in vivo because they lack sensitivity and/or specificity or require invasive methods (Dalle-Donne, 2006). Furthermore reactive oxygen species are generally too reactive and/or have a half-life too short to allow direct measurement in cells/tissues or body fluids. Because molecular products formed from the reaction of reactive oxygen species with biomolecules are generally considered more stable than reactive oxygen species themselves, most commonly reactive oxygen species have been tracked by measuring stable metabolites and/or concentrations of their oxidation target products, including lipid peroxidation end products and oxidized proteins (Davies *et* al., 1999; Dalle-Donne, 2003; Haliwell and Whiteman, 2004).

2.11 VITAMIN C

Vitamin C (Ascorbic Acid) is a powerful water soluble antioxidant and reducing agent (Terashito and Imamuna, 2005). It is an essential vitamin (Abdul-Razzak, 2012), required for multiple biological functions (Halliwell, 2001). Ascorbic acid is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not by humans, non-human primates and guinea pigs. These species do not have the enzyme gulonolactone oxidase, which is essential for synthesis of the ascorbic acid immediate precursor 2-keto-l-gulonolactone. The DNA encoding for gulonolactone oxidase has undergone substantial mutation, resulting in the absence of a functional enzyme (Nishikimi *et al*., 1994; Nishikimi and Yagi, 1996). Consequently, when humans do not ingest vitamin C in their diets, a deficiency state occurs with a wide spectrum of clinical manifestations. Clinical expression of vitamin C deficiency, scurvy, is a lethal condition unless appropriately treated. Thus, humans must ingest vitamin C to survive. (Sebastian *et al.*, 2003)

2.11.1 Sources of Vitamin C

Ascorbic acid is obtained mainly from a diet of fruits and vegetables (Padoyotty *et a*.,2003).Five servings of fruits and vegetables contain approximately 200 mg of vitamin C (Sebastian *et al.*, 2003). Most of the plants and animals synthesize ascorbic acid from D-glucose or D-galactose. in humans ascorbic acid has to be supplemented through food and or as tablets (Naidu, 2003).

2.11.2 Dietary Recommendations of Ascorbic Acid

The new average daily intake level that is sufficient to meet the nutritional requirement of ascorbic acidDietary Reference Intake (DRI) for vitamin C is 75 mg per day for women and 90 mg per day for men (Faiella, 2005).Consumption of 100 mg/day of ascorbic acid is found to be sufficient to saturate the body pools (neutrophils, leukocytes and other tissues) in healthy individuals. Based on clinical and epidemiological studies it has been suggested that a dietary intake of 100 mg/day of ascorbic acid is associated with reduced incidence of mortality from heart diseases, stroke and cancer. However, stress, smoking, alcoholism, fever, viral infections cause a rapid decline in blood levels of ascorbic acid (Naidu, 2003). Smoking is known to increase the metabolic turnover of ascorbic acid due to its oxidation by free radicals and reactive oxygen species generated by cigarette smoking (Frei *et al*., 1981). It has been suggested that a daily intake of at least 140 mg/day is required for smokers to maintain a total body pool similar to that of non-smokers consuming 100 mg/day (kallner *et al.*, 1981). Based on latest literature reports, it has been recommended that the DRI for ascorbic acid should be 100–120 mg/day to maintain cellular saturation and optimum risk reduction of heart disease, stroke and cancer in healthy individuals (Carr & Frei., 1999). Ascorbic acid is a labile molecule; it may be lost from foods during cooking/processing even though it has the ability to preserve foods by virtue of its reducing property. Synthetic ascorbic acid is available in a wide variety of supplements viz., tablets, capsules, chewable tablets, crystalline powder, effervescent tablets and liquid form (Naidu, 2003). Vitamin C is an over- the-counter drug that is intensively advertised as a health promoter. It is available to the public in doses beyond the current DRI (Abdul azziz, 2012). Buffered ascorbic acid and esterfied form of ascorbic acid as ascorbyl palmitate is also available commercially. Both natural and synthetic ascorbic acid are chemically identical and there are no known differences in their biological activities or bio-availability (Naidu, 2003).

2.11.3 Vitamin C Antioxidant Function

Vitamin C is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule (Sebastian *et al.,* 2003). Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. However, by the very nature of this reaction, vitamin C itself is oxidized in the process. It is noteworthy that when vitamin C donates electrons, they are lost sequentially. The species formed after the loss of one electron is a free radical, semidehydroascorbic acid or ascorbyl radical (Sebastian *et al.,* 2003). As compared to other free radicals (a species with an unpaired electron), ascorbyl radical is relatively stable with a half-life of 10^{-5} seconds and is fairly unreactive (Buettner *et al*., 1993; Sebastian *et al.,* 2003).

This property explains why ascorbate may be a preferred antioxidant (Sebastian *et al.,* 2003). In simple terms, a reactive and possibly harmful free radical can interact with ascorbate. The reactive free radical is reduced, and the ascorbyl radical formed in its place is less reactive. Reduction of a reactive free radical with formation of a less reactive compound is sometimes called free radical scavenging or quenching. Ascorbate is therefore a good free radical scavenger due to its chemical properties (Buettner *et al*., 1993; Sebastian *et al.,* 2003), and is a potent water soluble antioxidant capable of scavenging/ neutralizing an array of reactive oxygen species viz., hydroxyl, alkoxyl, peroxyl, hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide, peroxynitrite at very low concentrations. In addition ascorbic acid can regenerate other antioxidants such as α-tocopheroxyl, urate and β-carotene radical cation from their radical species. (Naidu, 2003) As an important dietary antioxidant, it significantly decreases the adverse effect of reactive species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis (Halliwell and Gutteride, 1999).It is well known that in the presence of redox-active iron, ascorbic acid acts as a pro-oxidant *in vitro* and might contribute to the formation of hydroxyl radical, which eventually may lead to lipid, DNA or protein oxidation. Thus, ascorbic acid supplementation in individuals with high iron and or bleomycin-detectable iron (BDI) in some preterm infants could be deleterious because it may cause oxidative damage to biomolecules However, no pro-oxidant effect was observed on ascorbic acid supplementation on DNA damage in presence or absence of iron (Naidu,2003).

2.11.4 Effect of Vitamin C on Heamatological Parameters

Adequate intake of vitamin C helps in the treatment of cardiovascular diseases (vasodilation and hypertension), (Ibitoroko *et al*, 2011). Ambali *et al*., (2010) in a study reported that pretreatment with vitamin C improved the RBC parameters depressed by chlorpyrifos. This may be due to the ability of the antioxidant vitamin to improve the absorption of iron from the gut (Iqbal et al., 2004) by facilitating the reduction of oxidized iron to its reduced form (Ambali *et al*., 2010) Treatment with vitamin C ameliorate the leukocytosis induced by toxins in some animal study. The reason for the apparent restoration of vitamin C on leukocytosis is not known for certain but may be due to its modulatory role on the immune cells. (Ambali *et al*., 2010). The combination of vitamin C and vitamin E help to reversed the haematoxic damage by increasing the haemoglobin in some toxicology studies (Ibitoroko *et al*, 2011)

Anemia during scurvy in mammals has been shown to be due to disturbances in iron metabolism particularly in its absorption, transfer from plasma to ferritin and distribution of the ferritin itself, loss of blood through hemorrhages. Vitamin C aids the absorption of iron, which is essential in erythropioesis (Kannall, 2014). Vitamin C has been shown, in a number of studies, to restore damges on RBC. The change from a normochromic, normocytic condition to a normochromic, macrocytic one, eventually leading to hypochromic, macrocytic in vitamin C-deficient fishes. The macrocytosis as a result of chronic deficiency is probably an adaptive response through an influx of immature erythrocytes from the hemopoietic tissues to the peripheral blood to make up for the reduced RBC number and decreased hemoglobin concentration (Wal, *et at*., 1987).

In another study on fishes with vitamin C deficiency induced from birth, the macrocytic condition was accompanied by a large number of developing red blood cells (reticulocytes) appearing in the peripheral blood. The erythropoietic studies during present investigation revealed a decrease in the earliest stages of erythropoietic development, that is small lymphoid hemoblast, indicating thereby inhibition of cell proliferation at the stem cell stage. On the other hand, there was an increase in the relative population of young and mature reticulocytes in the hemopoietic tissues indicating failure of hemoglobin incorporation at this stage and consequent blocking of further development (Wal, *et at*., 1987).

2.11.5 Other Functions of Vitamin C

Vitamin C is an essential nutrient that is required by humans in a small amount, but conclusive medical evidence for the use and safety o f a mega-dose of vitamin C in treatment of specific conditions is still controversial (Abdul-Razak, 2012). Vitamin C has been implicated in a wide range of related and unrelated aspects of health and disease. Considerable information has been presented during the last thirty to forty years describing the beneficial effect of a high dose of vitamin C (200 mg to 10 gm). There is a strong supported movement for using large doses of vitamin C in an attempt to obtain specific therapeutic effects. Several researchers have used high doses of vitamin C is the treatment of cancer (Abdul-Razak, 2012), common cold (Borts, 2008; Douglas, 2005; Douglas *et al*., 2004), heart diseases (Douglas *et al*., 2000), acute pancreatitis (Cherubini *et al*., 2005), Helicobacter pylori infection (Du *et al*., 2003) and wound healing (Jaros *et al*., 1998). Sebastian *et al*,. (2003) stated that the only proven function of vitamin C is the prevention of scurvy. Intake of as little as 10 mg/day of vitamin C will prevent scurvy. At a dose of 200 mg of vitamin C, steady state plasma concentrations are about 70 _M. Tissue vitamin C concentrations are higher than that of plasma. Similar to plasma, tissue vitamin C concentrations also change with vitamin C intake. Tissues, however, saturates before plasma, at a vitamin C intake of 100 to 200 mg/day. The accumulated vitamin C in plasma and tissues is much more than that necessary to prevent scurvy and may simply serve as a reservoir of the vitamin. Thus, exquisite mechanisms exist to avidly accumulate and tightly regulate plasma and cellular vitamin C concentrations. When adequate vitamin C is available in the diet, these mechanisms keep plasma vitamin C concentrations at levels that are approximately an order of magnitude higher than that necessary to prevent scurvy. These complex mechanisms, which appear to be well conserved, are likely to subserve some important function.

Low but non-scorbutic plasma vitamin C concentrations produce fatigue. These fact suggest that large (more than the amount needed to prevent scurvy) intake of vitamin C and high plasma and tissue concentrations may have clinical benefits. Similar to the proposed study of its antioxidant benefits, these benefits may be demonstrated in normal volunteers using vitamin C depletion-repletion study design to safely achieve extremes of plasma and tissue vitamin C concentrations (Sebastian *et al.*, 2003).

As an electron donor, vitamin C acts as a cofactor for several enzymes that are involved in hydroxylation reactions including collagen synthesis, production of certain hormones, neurotransmitters, and bile salts. Besides that, ascorbic acid has an important role in maintaining a proper immune system(Padayotty *et al.*, 2003; Roifes *et al.*, 2009). Ascorbic acid supplementation is found to facilitate the dietary absorption of iron. The reduction of iron by ascorbic acid has been suggested to increase dietary absorption of non-heme iron. The reduced iron may be the preferred form for intestinal absorption (Hallberg, 1995; Lynche, 1997).

Ascorbic acid plays an important role in the maintenance of collagen which represents about one third of the total body protein. It constitutes the principal protein of skin, bones, teeth, cartilage, tendons, blood vessels, heart valves, inter vertebral discs, cornea and eye lens. Ascorbic acid is essential to maintain the enzyme prolyl and lysyl hydroxylase in an active form. The hydroxylation of proline and lysine is carried out by the enzyme prolyl hydroxylase using ascorbic acid as co-factor. Ascorbic acid deficiency results in reduced hydroxylation of proline and lysine, thus affecting collagen synthesis (Naidu, 2003). On the other hand, possible potential harmful effects of a high dose of vitamin C may also be present. The combination of chronic psycho-social stress and vitamin C causes liver injury that is correlated with the presence of histological lesions. The effects of chronic stress and vitamin C on the liver were dependent on the dosing level of vitamin C, where only high dose vitamin C (500 mg/kg/day) was associated with liver injury during chronic stress conditions (Abdul-Razak, 2012).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIAL

3.1.1Chemicals

All chemicals used in this study were of analytic grade. Lead acetate(May and Baker. England), vitamin C(Emazor, Lagos, Nig),Chloroform (Sigma Chemical Co. USA), and Picric acid reagent (BDH Ltd Poole, England), NWLSSTM Malondialdehyde assay kit, NWLSSTM Superoxide dismutaseactivity assay kit, NWLSSTM Glutathione peroxidaseassay kit.

3.1.2 Equipment

Animal cages, mortar and pestle, digital weighing scale, scissors, syringes (1 ml and 5mll), oral gavage canula, bottles, EDTA containers, anti-coagulant free containers, filter paper, cotton wool.

3.2ANIMAL MANAGEMENT

Twenty(20)healthyalbino wistar rats of both sex weighing 150gm to 200gm were obtained from animal house, Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were fed with pellets made from grower's mash and drinking water *ad labium*. The animals were kept in clean cages and the floors of the cages were layed with saw dusts, cleaned and replaced every three days. The study was conducted in accordance with the US guideline as contained in the National Institute of Health guide for the care and use of laboratory animals (NIH publication No. 18-23, 1985).

3.3 METHODS

3.3.1 EXPERIMENTAL DESIGN

Animals were divided into experimental groups (n=5) exposed orally to chemicals daily for 3 weeks

Group 1- Control,were given distilled water 0.2ml/kg

Group 2- Were given lead acetate 250mg/kg (Barber *et al*.,2011) daily for 3weeks(AbdelAziz, 2010).

Group 3- Were given 100mg/kg of Vitamin C (Patrick, 2006) for 3 weeks.

Group 4-Were be given 250mg/kg of lead acetate and 150mg/kg of vitamin C daily for 3 weeks.

3.3.2Sample Collection

Blood samples was collected from the heart by cardiac puncture after euthanizing the animals with chloroform anaesthesia into vacutainer tubes coated with Ethylene diamine tetraacetic acid (EDTA) for hematological analysis. A second blood fraction was collected without anticoagulant in centrifuge tubes allowed to clot and the serum separated by centrifugation for determination of Lipid peroxidation and antioxidant enzyme activities.

3.3.3Determination of Haematological Parameters

Three (3)ml of blood sample were collected by cardiac puncture and red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV) , mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV) Platelets count were determined usingautomated cell counter, sysmsex automated hematological analyzer (KX-21N, Sysmex coporation Koba-Japan) (Halim *et al.*,

2011) at Anti-Retroviral Therapy Laboratory, Nassarawa Center, Ahmadu Bello University Teaching Hospital, Shika and value were recorded.

3.3.4Biochemical Analysis

Blood samples werecentrifuged at 3000 rpm, using a centrifuge (Centurion model GP (CD295–30) at 4 °C for 10 min to obtain clear serum.

Determination of Lipid Peroxidation

Plasma malondialdehyde (MDA) an indicator for lipid peroxidation was measured using Malondialdehyde Assay kits fromNorthwest Life Sciences Specialities (NWLSS) product NWK-MDA01.

10 µL BHT Reagent was added to microcentrifuge vial followed by 250 µL of sample to vial and 250 µL Acid Reagent to vial and then 250 µL TBA Reagent to vial. Microcentrifuge vial was vortex vigorously (5-count) and incubated for 60 minutes at 60°C. The vial was centrifuge at 10,000x*g* for 2-3 minutes and the reaction mixture was transferred to cuvette. Spectra was recorded from 400-700 nm. 3rd derivative analysis was performed.

Assessment of Antioxidant Enzyme Activity

Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined from plasma using NWLSTH NWK-SOD02 assay method described by manufacturer.

920 µL of assay buffer was added to each cuvette for assay. 40 µL of Sample was be added, mixed andincubated for two (2) minutes.40 µL Hematoxylin reagent was added to start the auto-oxidation reaction.Absorbance was recorded at 560 nmevery 10 seconds for 5 minutes.

Glutathione Peroxidase

NWLSSTH Glutathione peroxidase assay kit was used in determining level of glutathione peroxidase activity using manufacturer's instruction by Standard Procedure for Microplate Assay All reagents were bought to room temperature. 50 μL of diluted sample was added to microplate wells. 50 μL of Working NADPH was added to each well and then 50 μL of Working H2O2 to each well.After 1 minute, A340 was monitored for 5 minutes with a recording interval of every 30 seconds. GPx activity was calculated from the net rate (Paglia and Valentine, 1967).

3.3.5Determination of Body Weight

Body weight changes of rats over a period of three weeks during drug administration was determined using a digital weighing scale. The body weights were recorded from day 0 to the 21^{th}day on termination of the chemical administration.

3.3.6Statistical Analysis

Result from the study were expressed as mean ± SEM. The differences between the groups was analyzed using SPSS Version 20, by one-way analysis of variance (ANOVA) followed by Tukeys post ho test. The values of $p<0.05$ was considered as significant (Duncan *et al.*, 1977).

CHAPTER FOUR

4.0 RESULTS

4.1 HAEMATOLOGICAL PARAMETERS

After three weeks of oral administration of 0.2ml/kg body weight of distilled water (control group), 250mg/kgbody weight of lead actate (group ii), 250mg/kg body weight of lead actate with 100mg/kg body weight of vitamin C (group iii), 250mg/kg body weight of lead actate with 150mg/kg body weight of vitamin C (group iv), result from haematological study shows a small increase in RBC count in group 2 which was not statistically significant ($p>0.05$)compared to control group. No statistially significant ($p>0.05$)changes was seen ingroups 3 and 4 in comparison with control group. A slight increase in RBC count was seen in group 4 compare to group 3 (table 4.1). Hb and PCV were slightly decrease in test groups compared to control group, the difference were not statistically significant ($p>0.05$). Value from group 4 was slightly higher compared to group 3 (Table 4.1). MCV show no significant difference in test groups in comparison with control group. Group 2 show slight decrease in comparison to control group which is not statistically significant. A slight decrease was recorded in groups 3 and 4 compared to control group. Group 4 shows slight increase in comparison with group 3 but all the differences were not statistically significant ($p>0.05$) (Table 4.1).

A slight decrease in MCH was recorded in group 2 compared to control group but not statistically significant ($p>0.05$). Values obtained from group 3 show a slight increase compared to group 2. Group 4 show a slight decrease compared to group 3 but not statistically significant ($p>0.05$). MCHC slightly decrease in group 2 compared to control group, group 3 and group 4 show slight decrease in comparison to control group, no statistically significant difference observed ($p>0.05$). Group 3 show slight increase in comparison to group 2. Values obtained from group 4 show slight increase compared to groups 2 and 3 but not statistically significant ($p>0.05$)(Table 4.1)

Table 4.1 show slight decrease in group 2 compared to control group in platelet count but no statatistically significant changes ($p>0.05$).Values obtained from group 3 and group 4 show slight decrease compared to control group but no statistically significant changes ($p>0.05$). MPV values obtained from test groups show slight increase in group 2 in comparison with control group. Group 4 show slight increase compared to group 2 and group 3. No statistically significant changes were recorded ($p>0.05$).

Table 4.1; Mean values of haematological parameters.

GROUPS	Haematological parameters							
	RBC (!10$^{6/\mu l}$)	HB (g/dl)	PCV (%)	MCV (f/l)	MCH (pg)	MCHC (g/dl)	PLT (10$^{3/\mu l}$)	MPV f/l()
Group 1 (Distilled water)	7.07±0.3^{ns}	13.00±0.6^{ns}	40.52±0.8^{ns}	57.54±0.2^{ns}	18.38±0.3^{ns}	31.87±0.5^{ns}	756.0±58.7^{ns}	7.46±0.2^{ns}
Group 2 (Lead 250mg/kg)	7.27±0.2^{ns}	12.32±0.6^{ns}	40.37±1.4^{ns}	55.50±1.1^{ns}	16.95±0.3^{ns}	30.47±0.2^{ns}	742.0±68.0^{ns}	7.60±0.2^{ns}
Group 3 (Lead 250mg/kg and vit c 100mg/kg)	6.50±0.3^{ns}	11.16±0.8^{ns}	35.90±1.8^{ns}	53.28±0.8^{ns}	17.16±0.6^{ns}	31.43±0.3^{ns}	722.0±59.0^{ns}	7.86±0.2^{ns}
Group 4 (Lead 250mg/kg and vit c 150mg/kg)	7.03±0.3^{ns}	11.60±0.9^{ns}	37.40±1.3^{ns}	55.40±0.9^{ns}	16.60±0.6^{ns}	31.14±0.3^{ns}	703.0±46.2^{ns}	7.56±0.3^{ns}

Effect of lead acetate and vitamin on haematological parameters after 3 weeks of administration. In all groups n=5. Value are considered not statistically significant (^{ns})compared to control. Results are expressed in mean ± SEM $p>0.05$. Tukeys post hoc test, ($^{ns\,-}$ not significant).

4.2 LIPID PEROXIDATION AND ANTIOXIDANT ACTIVITY

Activities of oxidative stress biomarker in plasma of control and test animals are shown in table 4.2 Activity of MDA, increased in group 2 compared to control group but dcreasesd in 3 and 4 compared to group 2. SOD and GPx decxreased in groups 2, 3 and 4 compared to control group but changes were not statistically significant ($p>0.05$).

Table 4.2; Mean values of oxidative biomarkers

GROUPS/TREATMENT	Oxidative stress biomarkers		
	MDA (µg/ml)	SOD (IU/L)	GPx (IU/L)
Group 1 (Distilled water)	1.52±0.1	1.60±0.2^{ns}	47.60±2.7^{ns}
Group 2 (Lead 250mg/kg)	2.22±0.1	1.54±0.3^{ns}	43.50±1.0^{ns}
Group 3 (Lead 250mg/kg and vit c 100mg/kg)	1.95±0.1^{ns}	1.52±0,2^{ns}	41.25±2.0^{ns}
Group 4 (Lead 250mg/kg and vit c 150mg/kg)	1.85±0.1^{ns}	1.24±0.1^{ns}	37.40±1.5^{ns}

Effect of lead acetate and vitamin C on bichemical enzymes after 3 weeks of administration. In all groups n=5. Value are considered not statistically significant (^{ns}) compared to control. Results are expressed in mean ± SEM $p>0.05$. Tukeys post hoc test, ($^{ns-}$ not significant).

4.3 EXPERIMENTAL OBSERVATIONS

Following the administration of leadacetate 250mg/kg, vitamin C 100mg/kg and 150mg/kg body weight, the following observations were made. Normal rat activity from week 0 to week 1 in all groups.. Normal activity in control group and slight decrease of activity in group 2,3 and 4 from week 1 to week 2. Normal activity in control group and much decrease in activity and sign of weakness in group 2, decreased activity in group 3 and 4 from week 2 to week 3.

4.4 RAT SURVIVAL AND BODY WEIGHT

During the treatment of animals 20% mortality was recorded in group 2 and group 3 in second and third weeks respectively. 90% survival rate was recorded at the termination of the study.

The mean body weight of the animals from week 0 to week three shows increase in control group. There was initial increase in animal body weight from week 0 to week 1 and then slightly decreased from week 2 to week 3 in group two animls. Animal from group 3 and group 4 also shows increase in mean body weight to a lesser extend compared to animals in control group. Mean value body weight of animals in control group shows the highest increase but all changes are not statistically significant (Table 4.3).

Table 4.3; Mean weightof animal.

GROUPS	Animal weight			
	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)
Group 1 (Distilled water)	159.8±4.8^{ns}	182.8 ±8.7^{ns}	199.8±8.7^{ns}	207.8±9.3^{ns}
Group 2 (Lead 250mg/kg)	171.7±7.8^{ns}	172.5±10.7^{ns}	168.7±7.6^{ns}	167.2±8.1^{ns}
Group 3 (Lead 250mg/kg and vit c 100mg/kg)	165±17.9^{ns}	167.0±11.4^{ns}	177.2±9.5^{ns}	179±9. 9^{ns}
Group 4 (Lead 250mg/kg and vit c 150mg/kg)	171.6±9.4^{ns}	175.7±5.4^{ns}	178.2±4.8^{ns}	180.2±4.6^{ns}

Effect oflead acetate and vitamin C on animal body weigh after 3 weeks of administration In all groups n=5. Value are considered not statistically significant (^{ns})compared to control. Results are expressed in mean ± SEM p>0.05. Tukeys post hoc test, ($^{ns-}$ not significant).

CHAPTER FIVE

5.0 DISCUSSION

An enormous amount of information is available on the effects of lead on human health. The effect of chronic exposure to low dose of lead on haematological parameters but very little is known about effect of short term exposure to lead on haematological parameters and biomarkers of oxidative stress.

Haematological parameters are rapid and detectable means of assessing different health conditions and alterations in the haematological parameters, may be used as indicators of disease, or stress in different animal species (Uboh *et al.*, 2012). In this study exposure to 250mg/kg body weight of lead acetate to adult albino rats for 3 weeks caused a slight increase in RBC and MPV. Increase in RBC in lead exposed rats observed in this study was previously reported by other researchers (Jacob *et al*., 2000; Iavicoli *etal.*, 2003; Golalipour et al., 2007). Iavicoli *et al*.(2003) observed that small increase of blood lead was associated with increased RBC count and also increased Hb and PCV levels, but in this study, Hb and PCV were slightly reduced while RBC count slightly increased in lead acetate treated rats. It may be due to the slight decrease in hemoglobin production because of lead induced disturbance of heme biosynthesis (Galalipour *et al*, 2007).Decrease in Hb, PCV, MCV, MCHC, PLT was observed in this study. Mean corpuscular haemoglobin (MCH) is a calculation of the average amount of oxygen-carrying haemoglobin inside a red blood cell while mean corpuscular volume (MCV) is a measurement of the average size of RBCs. MCH and MCV are both slightly decreased in lead test groups compared to control in this study indicating shrink in size of RBCs and onset of microcytic anemia which could be due to onset of iron deficiency. Anaemia: the condition of having less than the normal number of red blood cells or less than the normal quantity of haemoglobin in the blood. The oxygen carrying capacity of the blood is, therefore, decreased leading to decreased MCH, MCV, and MCHC. Mean corpuscular hemoglobin concentration (MCHC) is a calculation of the average concentration of hemoglobin inside a red cell. Decreased MCHC values (hypochromia) are seen in conditions where the hemoglobin is abnormally diluted inside the red cells, such as in iron deficiency anaemia. MCHC was slightly decreased in lead acetate treated group compared to control group but administration of vitamin C reserved the slightalterations induced by lead acetate in group 3 and 4 in this study. Golalipour *et al*., (2007) reported in a previous study that may be bone marrow could overcome lead toxicity because of exposure which was not at high dose, but it could suppressed the production of Hb. Alteration in Hb level might result in reduced oxygen transfer by RBCs. Decrease in MCV and MCH levels after administration of lead acetate in this study was in agreement with several studies (Falke and Zwennis, 1990; Antonowicz *et al.*, 1991; Noori *et al., 2003*.; Golalipour *et al*., 2007).

Oxidative stress occurs when generation of reactive oxygen species (ROS) exceed the capacity of antioxidant defense mechanisms leading to both an increase in oxidative processes and a decrease in antioxidant defenses (Xu *et al*., 2008) Lead induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning (Xu *et al*., 2008). Although, the mechanism by which lead induce oxidative stress is not fully understood, a large number of evidences indicate that multiple mechanism balance between reactive oxygen metabolites and antioxidant defense results in oxidative stress (Gibananada and Hussain, 2002).

The result obtained from this study indicate an increase in plasma malondialdehyde (MDA) level an indicator of lipid peroxidation, after exposure to lead acetate 250mg/kg body weight. Lipid peroxidation is a major event induced by oxidative stress. Free radicals generate a cascade of reactions which induce lipid peroxidation (Kovacheva and Ribarov, 1995) leading to a range of enzymatically damaging consequences (Kovacheva and Ribarov, 1995) including membrane disorganization. Thus, lipid peroxidation is considered a serious consequence of free radical toxicity which may even cause cellular death (Urso and Clarkson, 2003). Increase in Lipid peroxidation seen in this study has been supported by a number of studies. Many *in vitro* and *in vivo* studies have showed that MDA increases with lead treatment (Yiin and Lin 1995; Shafig-ur-Rehman *et al.* 1995). Studies on lead exposed animals showed increased lipid peroxidation or decrease in antioxidant defense mechanism (Adegbesan and Adenuga, 2007; Bokara *et al*., 2008). A number of researchers have also shown enhanced rate of lipid peroxidation in lead exposed rats (Yiin and Lin, 1995; Adegbesan and Adenuga, 2007; Bokara *et al*., 2008).

SOD is considered a primary enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide and it is involved in direct elimination of ROS. SOD plays an important role in protecting the cells against the toxic effects of O2- by catalyzing its dismutation reactions (Halliwell and Gutteridge, 1989). SOD keeps the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress (Fridovich, 1997). Various findings demonstrated that lead has inhibitory effects ion superoxide dismutase (Soltanianejad *et al*., 2003; Vaziri et al., 2003). Some studies showed decreased activities of SOD (Chaurasia and Kar, 1997a; Chaurasia and Kar, 1997b) Superoxide anions (O2-) itself directly affects the activity of peroxidise by affecting intracellular enzymes (Ghosh and Myers, 1998) SOD was found to be decreased in the treated animal in various studies (Sharma et al 2011b Sharma *et al*., 2011c). A decrease in SOD was explained by direct blocking action of the metal on –SH group of the enzyme (Kasperczyk *et al*., 2004).

The antioxidant enzyme defence system is made up of free radical scavengers including glutathione peroxidase (GP_X). Glutathione peroxidase (GPx) level of treatment groups were slightly decrease compared to control group. This suggests that the antioxidant activity of this enzyme was slightly altered. Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (Sharma et al., 2011a; Sharma *et al*., 2011b). Administration of lead acetate enhanced oxidative stress, manifested by increase in MDA level and slightly suppressed the activity of antioxidant enzymes (SOD, GPx) in blood plasma of rats.

Ascorbic acid (Vitamin C) has been studied extensively in modulating lead induced alterations.. Ascorbic acid is known to have number of beneficial effects against lead exposure (Flora and Tandon, 1986). There has been considerable debate concerning the relationship between vitamin C nutritional status and heavy metal body burden in lead induced exposure. Early reports found that vitamin C might act as a possible chelator of lead, with similar potency to that of EDTA (Bassem, 2009). Most of the studies carried out to assess the effect of vitamin C on lead exposure used very high doses of vitamin C or administrate vitamin C concurrent with other vitamins such as vitamin E and vitamin D to reserve effect of lead. The effect of high dose vitamin C (1000mg/kg-2000mg/kg body weight) on lead levels has been clarified by studies but very little has been done to access the effect of vitamin C in low dose on lead induced alterations.In some animal studies with higher doses of vitamin C, Vitamin C intervention normalized blood ALAD levels and resulted in a significant decrease in blood lead level and hence reversing the effect of lead (Patrick, 2006b).In a study assessing the mechanism of vitamin

C's lead-lowering capacity blood lead levels were effectively loweredlead with high dose of vitamin C administrated at a longer term (Patrick, 2006b).The effect of vitamin C on lead levels has been seen in some studies show that ascorbic acid decreases intestinal absorption of lead (Patrick, 2006b).Findings from this study did not indicate amelioration of heamatological parameters after administrating lead acetate concurrent with vitamin C 100mg/kg body weight and lead acetate concurrent with vitamin 150mg/kg body weight. The lead-lowering effect of vitamin C has not been proven effective in exposure. Neither treatment affected lead levels or lead metabolism (Patrick 2006b). In one animal studies, the combined administration of lead-exposed rabbits with low dose Vitamin C did not confer significant protection against lead toxicity and from the findings better results were obtained when treatment started after complete stoppage of lead administration (Laila *et al.*, 2005). The disappearance of the effect of these vitamins on the haematological parameters and antioxidantenzymes could be due to decreased absorption of vitamin C as a result of low concentration of vitamin C or due to a maximum uptake of vitamin C by other peripheral tissues such as the liver and lungs at these concentrations used.

Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain ofreactions (Joseph *et al.*, 2009), that damage living cells. Antioxidants terminate chain reactions through the removal of free radical intermediates and inhibition of other oxidation reactions (Sies, (1997).Vitamin C acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead (Flora and Tandon, 1990).In this study the SOD and GPx activity were not reserved in lead acetate and vitamin C treated groups, however reversed the activity of MDA, by ameliorating the activities of MDA, vitamin C offered protection of cells against oxidative stress that may be by scavenging free radicals.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

After three weeks of administration,animals were sacrificed and blood samples collected via cardiac puncture. Haematological parameters were analysed which include RBC, WBC,Hb, MCV, MCH, MCHC, MPV, biochemical parameters which include MDA, SOD and GPx were also analysed. The result indicated no significant changes in haematological parameters, however, RBC count slightly increased in lead acetate treated rats which could be due to the slight decrease in hemoglobin production because of lead induced disturbance of heme biosynthesis.Decrease in Hb, PCV, MCV, MCHC, PLT was also observed which indicate onset of microlytic anaemia, however, the effect of lead on haematological parameters was not reversed by vitamin C administration concurrent with lead acetate. The lack of ameliorative effect of vitamin C on haematological parameters and antioxidant enzymes SOD and GPx could be due to low concentration of vitamin C a result of low dose used in the study, hence decreased absorption of vitamin C by RBC or due to a maximum uptake of vitamin C by other peripheral tissues. The effect of lead on antioxidant system was reflected in the increase of MDA, SOD and GPx were slightly decreased. Lipid peroxidation is considered a serious consequence of free radical toxicity, free radicals generate a cascade of reactions which induce lipid peroxidation. The increases in MDA level indicate oxidative stress. However, the doses of vitamin C used in this study did not ameliorate the alterations caused by the effect of lead on haematological parameters but reserved the effect of lead on MDA, the effect of lead on SOD and GPx were not reversed by vitamin C supplement.

6.2 CONCLUSION

Result obtained on the effect of vitamin C and short term low lead exposure on haematological parameters under the present investigation generally show no statistically significant changes.In conclusion the present study showed that lead acetate induced hematological changes were not significant after short term exposure and dose of vitamin C administrated did not effect in the improvement of impaired parameters affected by lead acetate exposure.

Low dose short term lead acetate administration induced oxidative stress by increasing the level of MDA, however SOD and GPx level did not show much effect. Dose of vitamin C used in this study doesnot have ameliorative effect on changes induced by low lead exposure on heamatological parameters and antioxidant enzyme activity but vitamin C reversed the effect of lead on lipid peroxidation.

6.3 RECOMMENDATION

i. Additional research priorties should include the effort to assess the anti oxidant effect of vitamin C at higher dose.
ii. Additional research should be directed towards assessing the effect of lead exposure at longer terms.
iii. Further work is needed to find the effective and safe intervention for lowering the lead exposure at the general population level to prevent consequence of exposure.
iv. Efficacy of specific intervention to keep blood lead concentration below reference level.

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