EFFECT OF FOOD CHAIN MEDIATED EXPOSURE TO CADMIUM AND ARSENIC ON SOME OXIDATIVE STRESS MARKERS IN RAT MODELS

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ABRAKA

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MARCH, 2017

CERTIFICATION

I declare that this research work was independently carried out by me **OKIEKE**, **Ogagaoghene** in the Department of Biochemistry, Faculty of Science Delta State University Abraka for the award of M.Sc. Degree in Biochemistry and has not been carried out by any one for the award of any Diploma or Degree.

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APPROVAL PAGE

This is to certify that this Research work was carried out by **OKIEKE, Ogagaoghene with** matriculation number **PG/12/13/213728** in the department of Biochemistry, Faculty of Science Delta State University for the award of Master of Science (M.Sc.) Degree in Biochemistry.

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Prof. S.O Asagba **Date**
(Supervisor)

_____ _____

Prof. N.J Tonukari **Date**
(*Head of Department*)

DEDICATION

This project work is dedicated to my parents Chief and Mrs. Peter Okieke and my Supervisor Prof. S.O. Asagba.

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I am most grateful to Almighty God, the creator of the universe and the giver of knowledge. I wish to express my sincere appreciation to my dear parents Peter and Onome Okieke for their love, care and support. I also thank my sibling Justice, Oghenero, Ufuoma, Weware, Kristie and Efemena for their understanding.

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ABSTRACT

The present study is aimed at investigating the comparative effects of food-chain mediated exposure to cadmium and arsenic on some biochemical indices of rats. Catfishes were divided into four groups: group A (control) were housed in fresh water, group B were housed in cadmium contaminated water, group C were housed in cadmium + arsenic contaminated water and group D were housed in arsenic contaminated water. All fishes received normal diet, kept for four weeks after which they were killed, oven dried and used as protein source in the diet for rats. The food-chain was mimicked by feeding various groups of male rats with their respective diets. Twenty eight (28) adult male rats were divided into four (4) groups of seven (7) rats each for this purpose: group A (control) was fed with normal diet containing non-metal exposed fish; group B (cadmium) were fed with diet containing cadmium contaminated fish; group C (arsenic) were fed diet containing arsenic contaminated fish; group D (cadmium + arsenic) were fed diet containing cadmium + arsenic contaminated fish. Administration was done for twelve weeks after which the animals were sacrificed, organs weighed and assays for AST, ALT, SOD, MDA and CAT activities were carried out. Results obtained suggest that cadmium is slightly more toxic than arsenic and both inhibit each other when administered together. The results obtained showed the consumption of arsenic and cadmium through the food chain for 3 months altered the activities of SOD, catalase and aminotransferases in plasma and tissue. Conversely, beside the testis, no significant change was observed in level of malondialdehyde in other organs of exposed rats. However, examination of the data obtained, show that in most cases, both metals appear to have antagonistic interaction when consumed together.

CHAPTER ONE

INTRODUCTION

1.0 Background to the Study

Human activities have evidently modified the global cycle of heavy metals and their compounds, including the toxic non-essential elements like cadmium (Cd), lead (Pb), and arsenic (As) (Clarkson, 1995). These heavy metals exhibits complex metabolism and are possibly among the most abundant and potential carcinogens (Roy and Saha, 2002). The humans are exposed to various types of these environmental contaminants at different stages of their life span; the majority of these are harmful.

There are around thirty chemical elements that play a pivotal role in various biochemical and physiological mechanisms in living organisms, and recognized as essential elements for life. In fact, for many food components, the intake of metal ions can be a double edged sword. Majority of the known metals and metalloids are very toxic to living organisms and even those considered as essential, can be toxic if present in excess (Mudgal *et al*., 2010). Concentrations of several toxic metal and metalloids have been largely increased as a result of human activities. They can disturb important biochemical processes, constituting an important threat for the health of plant and animals. Plants and animals absorb these elements from soils, sediments, and water by contact with their external surfaces, through ingestion and also from inhalation of airborne particles and vaporized metals (Mudgal et al., 2010; Madaan and Mugdal, 2009). The requirement for ingestion of trace metals such as Fe and Cu ions to maintain normal body functions such as the synthesis of metallo-proteins is well established. However, cases of excess intake of trace metal ions are credited with pathological events such as the deposition of iron oxides in Parkinson's disease (Candelaria *et al*., 2006). In addition to aiding neurological depositions, these redox active metal ions have been credited with enhancing oxidative damage, a key component of chronic inflammatory disease (Umanzor et al., 2006) and a suggested initiator of cancer (González-Cortijo et al., 2008). As inflammation is a characteristic feature of a wide range of diseases, further potential pathological roles for metal ions are emerging as exemplified by premature ageing (Mazzio and Soliman, 2009).

For the maintenance of health, a great deal of preventative measures is in place to avoid ingestion of potentially toxic metal ions. From monitoring endogenous levels of metal

ions in foods and drinks to detecting contamination during food preparation, European countries spend significant resources to avoid metal intake by the general population (González-Cortijo et al. 2008; Hida *et al*., 2000; Hida *et al*., 2002). From a therapeutic viewpoint, considerable research and development efforts are being exerted to decorporate metal ions from the body.

Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, fertilizer industries, and cigarette smoke. Cd is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues (Stohs and Bagchi, 1995). Cd shows various mechanisms of toxicity in particular species under different experimental conditions (Iscan et al., 1994; Žikić et al., 1996; Waisberg et al., 2003). Cd in soil and water is taken up by plants and is concentrated and transferred to upper links of the food chain, including humans (WHO, 1995; Satarug et al., 2003). Due to the long biological half-life of Cd (i.e., 10-30 years) its accumulation in the body can increase the risk of toxicity (Sugita and Tsuchiya, 1995). The principal determinants of human Cd exposure are smoking habits, diet and to a certain extent, occupational exposure. According to WHO (Asharaf, 2011) one cigarette (containing 0.5 – 3 μg Cd per gram of tobacco) can result in up to 3 μg daily Cd absorption via the lungs. Chronic exposure to these low doses of Cd causes neuroendocrine and neurobehavioral disturbances in animals and humans (Viaene et al., 2000; Lafuente et al., 2003; 2004; 2005; Leret et al., 2003).

The effects of Cd in high concentrations have been attributed to an excesive increase in reactive oxygen species (ROS) in cells. Such an effect is particularly evident in kidney and liver and has been also demonstrated in vitro in neurons (Lopez et al., 2006) and glial cells (Yang et al., 2007). ROS play a dual role in biological systems, since they can be either harmful or beneficial to cells (Valko et al., 2006). Beneficial effects of low amounts of ROS involve physiological roles in cellular responses to noxa, as for example in defense against infectious agents and in the function of a number of cellular signaling systems. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids and membrane proteins and nucleic acids (oxidative stress). While some metals, like iron, copper, chromium, vanadium or cobalt undergo redox-cycling reactions, a second group of metals including Cd, mercury and nickel cause toxicity mainly by depleting glutathione and by binding to sulfhydryl groups of proteins (Wright and Baccarelli, 2007). Metal-

mediated formation of free radicals causes various modifications to DNA bases and may alter calcium and sulfhydryl homeostasis.

It has been demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Waisberg et al., 2003). Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Shaikh et al, 1999; Casalino et al., 2002; Waisberg et al., 2003).

Arsenic (As) is a common environmental contaminant widely distributed around the world. Human exposure to this metalloid comes from well water and contaminated soil, from fish and other sea organisms rich in methylated arsenic species, and from occupational exposure (Rodrıguez et al., 2003). Arsenic poisoning is second to lead as the most frequently reported heavy metal toxicant. Inorganic Arsenic is incorporated into pesticides, which are the most common sources of arsenic poisoning (Singh et al., 2007). The incidence of fluoride, arsenic and iron in water has been reported in isolated pockets of India (Central Ground Water Board Report, 2010). Use of contaminated water is a serious public health issue as ground water is used without any kind of treatment (ATSDR, 1993). Arsenic exposure causes both acute and chronic toxicity in human. Human arsenic exposure is related to severe health problems such as skin cancer, diabetes, liver, kidney and CNS disorders. It also causes many other toxic effects (Jolliffe et al., 1991; WHO, 1981; Pershagen, 1983).

1.2 Statement of Problem

The negative effect of cadmium and arsenic has been well elucidated by various studies. Their high level of abundance in the environment has made them one of the most risky contaminants exposed to man through food and many other substances. In most of the available studies, the natural route of entry of metals to humans is not taken into account. In such studies the metals are either added directly to food or drinking water of the animals or they are introduced into animal by intramuscular or intraperitoneal injection. Thus the findings from these studies may not be reliable since these metals are expected to be biotransformed in plants or lower animals before they are taken in by man.

1.3 General Aims and Objectives of the study

The present study is aimed at investigating the effects of food-chain mediated exposure to cadmium and arsenic on some biochemical indices of rats.

1.3.1 Specific Objectives

The specific objectives of the study are to determine the following parameters in rats exposed to cadmium and arsenic via the food chain:

i. Weight gain and organ/body weight ratio.
ii. Activities of catalase and superoxide dismutase as well as lipid peroxidation in organs/tissues.
iii. Plasma and tissue aminotransferase activities.

1.4 Significance of Study

The potential effect of chronic and acute exposure to arsenic and cadmium has been well defined in animal and human models. The mechanism through which cadmium and arsenic act to cause deleterious effects has been linked to impaired antioxidant metabolism and oxidative stress may play a role. However, the toxicity of metals via the food chain has not been properly addressed and this underscores the importance of the present study.

1.5 Justification of the Study

The present study will provide scientific evidence on the role of the food-chain in the toxicity of metals such as cadmium and arsenic

1.6 Scope of the Study

Study is an experimental investigation on the comparative effect of cadmium, arsenic and a combination of arsenic and cadmium on enzyme activities (ALT, AST, SOD, Catalase) and lipid peroxidation in the tissues (liver, kidney, heart, testes prostrate, and brain) and plasma of rats.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 Cadmium

Cadmium is a chemical element with symbol Cd and atomic number 48. This soft, bluish-white metal is chemically similar to the two other stable metals in group 12, zinc and mercury. Like zinc, it prefers oxidation state +2 in most of its compounds and like mercury it shows a low melting point compared to transition metals. Cadmium and its congeners are not always considered transition metals, in that they do not have partly filled d or f electron shells in the elemental or common oxidation states. The average concentration of cadmium in the Earth's crust is between 0.1 and 0.5 parts per million (ppm) (Lide, 2005). It was discovered in 1817 simultaneously by Stromeyer and Hermann, both in Germany, as an impurity in zinc carbonate.

Cadmium makes up about 0.1 ppm of the Earth's crust. Compared with the more abundant 65 ppm zinc, cadmium is rare (Wedepohl, 1995). No significant deposits of cadmium-containing ores are known. Greenockite (CdS), the only cadmium mineral of importance, is nearly always associated with sphalerite (ZnS). This association is caused by the geochemical similarity between zinc and cadmium which makes geological separation unlikely. As a consequence, cadmium is produced mainly as a byproduct from mining, smelting, and refining sulfidic ores of zinc, and, to a lesser degree, lead and copper. Small amounts of cadmium, about 10% of consumption, are produced from secondary sources, mainly from dust generated by recycling iron and steel scrap (Fleischer et al., 1980; Fthenakis, 2004). Cadmium occurs as a minor component in most zinc ores and therefore is a byproduct of zinc production. It was used for a long time as a pigment and for corrosion resistant plating on steel while cadmium compounds were used to stabilize plastic. The use of cadmium is generally decreasing due to its toxicity, it is specifically listed in the European Restriction of Hazardous Substances (Morrow, 2010) and the replacement of nickel-cadmium batteries with nickel-metal hydride and lithium-ion batteries. One of its few new uses is in cadmium telluride solar panels. Although cadmium has no known biological function in higher organisms, a cadmium-dependent carbonic anhydrase has been found in marine diatoms.

Although cadmium usually has an oxidation state of +2, it also exists in the +1 state. Cadmium and its congeners are not always considered transition metals, in that they do not have partly filled d or f electron shells in the elemental or common oxidation states (Cotton, 1999). Cadmium burns in air to form brown amorphous cadmium oxide (CdO); the crystalline form of this compound is a dark red which changes color when heated, similar to zinc oxide. Hydrochloric acid, sulfuric acid and nitric acid dissolve cadmium by forming cadmium chloride (CdCl2), cadmium sulfate (CdSO4), or cadmium nitrate (Cd(NO3)2). The oxidation state +1 can be reached by dissolving cadmium in a mixture of cadmium chloride and aluminium chloride, forming the Cd22+ cation, which is similar to the Hg22+ cation in mercury(I) chloride (Martelli *et al*., 2006).

$$Cd + CdCl_2 + 2AlCl_3 \longrightarrow Cd_2(AlCl_4)_2$$

The structures of many cadmium complexes with nucleobases, amino acids and vitamins have been determined (Ashrav, 2012).

2.1.1 Properties of Cadmium

Cadmium is a soft, malleable, ductile, bluish-white divalent metal. It is similar in many respects to zinc but forms complex compounds (ATSDR, 2011). Unlike other metals, cadmium is resistant to corrosion and as a result it is used as a protective layer when deposited on other metals. As a bulk metal, cadmium is insoluble in water and is not flammable; however, in its powdered form it may burn and release toxic fumes (IARC, 1976; Ros & Slooff, 1987; Ware, 1989).

Cadmium generally occurs in small quantities associated with other metals, particularly zinc. The atomic weight of cadmium is 112.41. Cadmium melts at 320.9°C, and boils at 767°C. The specific gravity of cadmium is 8.65. The most common valence is 2. Cadmium forms a number of salts. The most common cadmium salts are cadmium sulfate and cadmium sulfide. The latter is a yellow pigment (Eisler, 2000).

2.1.2 Human Exposure to Cadmium

Humans are exposed to cadmium from all environmental media including air, drinking water, cigarette smoke, and food. Cigarette smoke and food are the major sources of exposure, with air and drinking water contributing lesser amounts

- **Air:** Cadmium is present in ambient air in the form of particles in which cadmium oxide is probably an important constituent (Friberg et al., 1986). Cigarette smoking increases cadmium concentrations inside houses. The average daily exposure from cigarette smoking (20 cigarettes a day) is 2–4 μg of cadmium (Ros and Slooff, 1987).
- **Soil:** Soil can become contaminated with cadmium from land disposal of cadmium wastes, from spreading of sewage sludge, and from the use of phosphate fertilizers (ATSDR, 1997).
- **Water:** Drinking water may become contaminated with cadmium due to its presence in solder used on metal pipes that carry drinking water. Cadmium in solder may be solubilized if the water is slightly acidic. (Hallenbeck, 1984). Contamination of drinking-water may also occur as a result of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing water heaters, water coolers and taps (Friberg et al., 1986).
- **Food:** Food is the main source of cadmium intake for non-occupationally exposed people. Crops grown in polluted soil or irrigated with polluted water may contain increased concentrations, as may meat from animals grazing on contaminated pastures (IARC, 1976). Animal kidneys and livers concentrate cadmium. Levels in fruit, meat and vegetables are usually below 10 μg/kg, in liver 10–100 μg/kg and in kidney 100–1000 μg/kg. In cereals, levels are about 25 μg/kg wet weight (Viana, 2011).
- **Cigarettes:** Cigarettes are the most significant source of cadmium exposure to adults who smoke (Bernard and Lauwerys, 1984). The average daily exposure from cigarette smoking (20 cigarettes a day) is 2–4 μg of cadmium (Ros and Slooff, 1987; Viana, 2011).

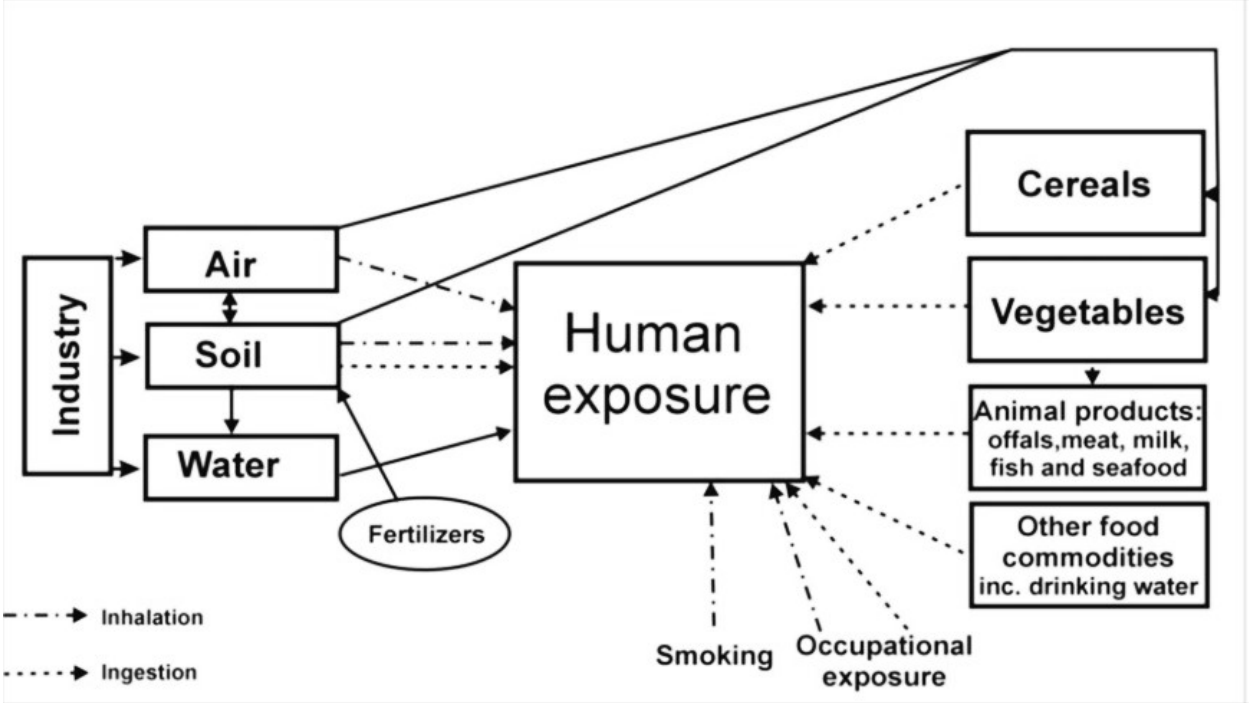


Fig. 1: Cadmium Human Exposure Route

Source: Faro et al., (2014)

2.1.3 Absorption of Cadmium

Cadmium metal and cadmium salts have low volatility and exist in air primarily as fine suspended particulate matter. When inhaled, some fraction of this particulate matter is deposited in the airways or the lungs, and the rest is exhaled. Large particles (greater than about 10 pm in diameter) tend to be deposited in the upper airway, while small particles (approximately 0.1 pm) tend to penetrate into the alveoli. While some soluble cadmium compounds (cadmium chloride and cadmium sulfate) may undergo limited absorption from particles deposited in the respiratory tree, the major site of absorption is the alveoli. Thus, particle size, which controls alveolar deposition, is a key determinant of cadmium absorption in the lung (Nordberg et al., 2007).

Based on comparison of cadmium body burdens in human smokers and nonsmokers, cadmium absorption from cigarettes appears to be higher than absorption of cadmium aerosols measured in animals (Nordberg et al., 2007). The chemical form of cadmium in cigarette smoke is likely to be similar to that produced by other combustion processes, primarily cadmium oxide aerosols. The greater absorption of cadmium from cigarette smoke

is likely due to the very small size of particles in cigarette smoke and the consequent very high alveolar deposition (Nordberg et al., 1985).

Most ingested cadmium passes through the gastrointestinal tract without being absorbed (Kjellstrom et al., 1978). Measurement of gastrointestinal absorption is complicated by the fact that not all of a dose initially retained in the gastrointestinal system can be considered to be absorbed, because some portion may be trapped in the intestinal mucosa without crossing into the blood or lymph (Foulkes, 1984). Thus, measures of whole-body cadmium retention may overestimate cadmium absorption (at least in the short-term). On the other hand, some absorbed cadmium may be excreted in urine or feces, so that retention may underestimate exposure. However, this underestimate is probably minor because excretion of absorbed cadmium is very slow.

2.1.4 Distribution of Cadmium

Cadmium is widely distributed in the body, with the major portion of the body burden located in the liver and kidney. Animals and humans appear to have a similar pattern of distribution that is relatively independent of route of exposure, but somewhat dependent on duration of exposure.

Cadmium was found in autopsy samples from nearly all organs of a worker extensively exposed to cadmium dust, with greatest concentrations in the liver, kidney, pancreas, and vertebrae (Friberg, 1950). In workers dying from inhalation of cadmium, lung-cadmium concentration is somewhat lower than liver or kidney cadmium concentration (Beton et al., 1966; Lucas et al., 1980; Patwardham and Finckh, 1976). The concentration of cadmium in the liver of occupationally exposed workers generally increases in proportion to intensity and duration of exposure to values up to 100 μg/g (Abernethy *et al*., 2010).

The concentration of cadmium in the kidney rises more slowly than in the liver after exposure (Gompertz et al., 1983) and begins to decline after the onset of renal damage at a critical concentration of 160-285 μg/g (Reels et al., 1981).

2.1.5 Metabolism of Cadmium

Regardless the route of exposure, Cadmium is efficiently retained in the organism and remains accumulated throughout life. The Cadmium body burden, negligible at birth, increases continuously during life until approximately the age of about 60-70 years from

which Cadmium body burden levels off and can even decrease. Cadmium concentrates in the liver and even more in the kidneys, which can contain up to 50 per cent of the total body burden of Cadmium in subjects with low environmental exposure. Accumulation of Cadmium in liver and kidney is due to the ability of these tissues to synthesize metallothionein, a Cd-inducible protein that protects the cell by tightly binding the toxic Cd2+ ion. The stimulation of metallothionein by zinc probably explains the protective effect of this essential element towards Cadmium toxicity. Because of its small size, metallothionein is rapidly cleared from plasma by glomerular filtration before being taken up by the proximal tubular cells. This glomerular-filtration pathway is at the origin of the selective accumulation of Cadmium in proximal tubular cells and thus in the renal cortex where this segment of the nephron is located. Cadmium does not cross easily the placental or the haemato-encephalic barriers, explaining its very low toxicity to the foetus and the central nervous system as compared with other heavy metals.

2.1.6 Excretion of Cadmium

Most cadmium that is ingested or inhaled and transported to the gut via mucociliary clearance is excreted in the feces. However, almost all excreted cadmium represents material that was not absorbed from the gastrointestinal tract. Most absorbed cadmium is excreted very slowly, with urinary and fecal excretion being approximately equal (Kjellstrom and Nordberg, 1978). Half-times for cadmium in the whole body of mice, rats, rabbits, and monkeys have been calculated to be from several months up to several years (Kjellstrom and Nordberg, 1985). Half-times in the slowest phase were from 20 to 50% of the maximum life span of the animal (Kjellstrom and Nordberg, 1985). In the human body, the main portion of the cadmium body burden is found in the liver and kidney and in other tissues (particularly muscle, skin, and bone).

2.1.7 Mechanism of Toxicity of Cadmium

Cadmium is toxic to a wide range of organs and tissues; however, the primary target organs of cadmium toxicity are the kidneys and liver. Organs such as the testis, pancreas, thyroid, adrenal glands, bone, central nervous system, and lung have also been studied for toxic effects.

Changes in the kidney due to cadmium toxicity have been well established. Chronic exposure to cadmium by the oral or inhalation routes has produced proximal tubule cell

damage, proteinuria (mainly low molecular weight proteins, such as β2-microglobulin), glycosuria, amino aciduria, polyuria, decreased absorption of phosphate, and enzymuria in humans and in a number of laboratory animal species. The clinical symptoms result from the degeneration and atrophy of the proximal tubules, or (in worse cases) interstitial fibrosis of the kidney (Stowe et al., 1972). Cadmium has been shown to perturb lipid composition and enhance lipid peroxidation (Gill et al., 1989). Depletion of antioxidant enzymes, specifically glutathione peroxidase and superoxide dismutase, has been proposed as the mechanism of cadmium's cardiotoxic effects (Jamall and Smith, 1985a), but subsequent studies showed that cardiotoxic mechanisms other than peroxidation are also present (Seifried *et al*., 2007). Cadmium has been shown to alter zinc, iron and copper metabolism (Wojchiech and Konrad, 2001) as well as selenium (Jamall and Smith, 1985).

Cadmium complexed with metallothionein from the liver can redistribute to the kidney (Dudley et al., 1985). When metallothionein-bound cadmium is transported to the kidney, it readily diffuses and is filtered at the glomerulus, and may be effectively reabsorbed from the glomerular filtrate by the proximal tubule cells (Foulkes, 1978). Exogenous metallothionein is thought to be degraded in lysosomes and released. This non-metallothionein-bound cadmium can then induce new metallothionein synthesis in the proximal tubule (Squibb et al., 1984).

Case studies indicate that calcium deficiency, osteoporosis, or osteomalacia can develop in some workers after long-term occupational exposure to high levels of cadmium. Bone lesions (accompanied by renal damage) have also been reported in aged and malnourished women living in Cd-polluted areas in Japan. (Itai-Itai disease). Effects on bone generally arise only after kidney damage has occurred and are likely to be secondary to resulting changes in calcium phosphorus and vitamin D metabolism. The daily intakes via food and exposure levels in air at which the bone effects occur are probably in the same range as those producing kidney affects.

Long-term occupational exposure to high levels of cadmium has been reported to cause emphysema and dyspnea in humans. The dose needed to produce these effects is higher than the dose needed to produce renal effects. Chronic inflammation of the nose, pharynx, and larynx has been reported in some studies. Anosmia is a frequent symptom in cadmium workers after prolonged exposure.

2.1.8 Role of Food Chain in Cadmium Toxicity

Certain compounds of cadmium (Cd) are highly toxic to humans. Cadmium is employed in several industrial processes such as: (a) protective coatings (electroplating) for metals like iron; (b) preparation of Cd-Ni batteries, control rods and shields within nuclear reactors and television phosphors. Some compounds are used as stabilizers for PVC. For non-smoking population the major exposure pathway is through food.

Depending on the dietary intake and the iron status, it has been estimated that a European or an American adult absorbs cadmium orally at an average rate varying between 1.4 and 25µg/day. (Vromman et al., 2008). The cadmium absorption from the gastrointestinal tract is usually about 5%. However it varies considerably and subjects with iron deficiency may absorb up to 10%. For a given individual, the absorption following oral exposure to cadmium is likely to depend on physiologic status (age; body stores of iron, calcium, and zinc; pregnancy history; etc.) and, also, on the presence and levels of ions (Zn) and other dietary components ingested with the cadmium.

Cadmium is readily taken up by plants. Potential source of cadmium toxicity is the use of commercial sludge for fertilizing agricultural fields. Some root crops (carrots and parsnip) and some leafy crops (lettuce and spinach) are able to accumulate more cadmium compared to other plant foods. Shellfish, leafy vegetables, rice, cereals, and legumes may contain relatively high levels of cadmium (Jarup et al., 1998; EU, 2009).

2.2.0 Arsenic

Arsenic (As) is a metalloid with an atomic weight of 74.92. It has several oxidation states, including elemental (0), trivalent (−3, +3) and pentavalent (+5). Inorganic arsenical products have been known since ancient times. Some compounds such as As_2S_2 (realgar) and As_2S_3 (orpiment) were used in ancient Greece and Rome as depilatories, cosmetics, therapeutic agents and poisons. During the Middle Age and Renaissance, arsenical compounds continued to be used as poisons in France and Italy until the discovery by Marsh in 1836 of a sensitive method to detect arsenic (Sabina al., 2005).

The main use of metallic arsenic is for strengthening alloys of copper and especially leads (for example, in car batteries). Arsenic is a common n-type dopant in semiconductor electronic devices, and the optoelectronic compound gallium arsenide is the most common semiconductor in use after doped silicon. Arsenic and its compounds, especially the trioxide,

are used in the production of pesticides, treated wood products, herbicides, and insecticides. These applications are declining, however (Sabina et al., 2005).

Arsenic is notoriously poisonous to multicellular life, although a few species of bacteria are able to use arsenic compounds as respiratory metabolites. Arsenic contamination of groundwater is a problem that affects millions of people across the world.

2.2.1 Properties of Arsenic

Arsenic has the chemical symbol As, the atomic number 33, and an atomic weight of 74.92. It is present throughout the Earth's crust at varying concentrations with an average con-centration of 1.7mg/kg (Robinson and Ayotte, 2006). The twentieth most abundant element in the Earth‘s crust, it is found in sedimentary, igneous, and metamorphic rocks. In sedimentary iron ores the average concentration is extremely high at 400 mg/kg (ISSI Consulting Group, et al. 2000).

Arsenic occurs in 5 different valence states:

- Arsine -3
- Elemental Arsenic 0
- Arsonium Metals +1
- Arsenites +3
- Arsenates +5

2.2.2 Exposure to Arsenic

The presence of arsenic and other elements (lead, chromium, mercury) in the biosphere results from processes inherent to human activity. Industrial metallic emissions into the atmosphere have a short lifetime (few days), but they travel long distances from their point of emission, accumulate on land and form sediment in lakes and rivers, where metals have a longer lifetime (from a few hundred years to several thousand years) (Ayres, et al., 2003). Arsenic is released from several anthropogenic sources such as mining waste, mineral debris, glass manufacture, computer chips, wood preservatives, insecticides, rodenticides, herbicides, and some agrochemicals. Before the advent of penicillin or other therapies, some organic arsenicals such as arsephenamine, salvarsan and their derivatives were used as anti-syphilitic agents (Saldivar and Soto, 2009). Some arsenic compounds are used to treat

trypanosomiasis (Huet et al., 1975) and acute promyelocytic leukemia (Gibaud and Jaoune, 2010). In recent years the use of arsenic trioxide as an effective chemotherapeutic agent has increased, particularly for the treatment of hematological malignancies (Antman, 2001). Its efficacy has been tested in vitro in a variety of cancer models, such as human glioma cells and murine tumors (Jennewein, et al., 2008), among others; providing insight into the pathways through which arsenic trioxide exerts its anti-tumoral effects (Wang, 2001). Evidence that arsenic is a micronutrient is based on animal studies showing that rats, chicks, hamsters, and goats have arsenic requirements of 25–50 mg/g of food (Jones, 2007; Nachman, 2007). It has been suggested that arsenic has an important role in the conversion of methionine to its metabolites taurine, labile methyl, and possibly the polyamines (Sakurai, 2003), and it might be necessary for the efficient utilization or metabolism of zinc (Kumagai et al., 2001). Oral exposure to arsenic occurs mainly through contaminated food, e.g. arsenic species found in fish and seafood such as arsenobetaine and arsenocholine which give rise to elevated blood arsenic concentrations (Kumagai and Sumi, 2001). Since high concentrations of arsenic in water and soil can be found in several places around the world (Smith et al., 2000), exposure may come from these sources as well. On the other hand, inhalation exposure occurs as a result of occupational exposure to agricultural pesticides or in mining–smelting activities (Rodriiguez, 2003;Schäfer et al., 1999). Of particular concern is the exposure of children in arsenic contaminated sites. Human oral exposure to arsenic has been mainly related to bladder, kidney, liver and skin cancer, while exposure through inhalation has been linked to lung cancer. Arsenic exposure is also associated with peripheral neuropathy and peripheral vascular disorders such as blackfoot disease, a disorder which results in gangrene of the lower extremities (Wang, 2001). From 1930 to 1940 several authors report one kind of fatal encephalitis in some syphilitic patients who were injected with arsphenamine or salvarsan compounds (Globus and Ginsburg, 1933; Osterberg and Kernohan, 1934; Roseman and Aring, 1941; Russell, 1937). More recently, acute arsenic exposure was reported to cause central nervous system (CNS) alterations (Beckett et al., 1986; Bolla-Wilson and Bleecker, 1987; Frank, 1976; Morton and Caron, 1989).

The effects of arsenic on nervous system function have received considerably less attention than its association to cancer, genotoxicity, and cellular disruption. The purpose of this review is to integrate the reports on possible interactions of arsenicals with the nervous system and to promote interest in exploring the mechanisms of arsenic neurotoxicity.

2.2.3 Absorption, Distribution and Metabolism of Arsenic

Studies performed in animals and humans demonstrate that arsenicals are absorbed by both oral and inhalation routes (ATSDR, 1988; Rodriguez 2003). Once absorbed, arsenic is stored in the liver, kidney, heart and lung, while lower amounts are present in muscle and neural tissue (Klaassen, 1996). Two to four weeks after arsenic ingestion, it is incorporated into the nails, hair, and skin by binding to keratin sulfhydryl groups (Rodr´ıguez, 2003). Transverse white striae (Mee's lines) in nails are indicative of arsenic exposure. The characteristic Mee's lines appear as single, solid, transverse white bands of about 1 or 2mm in width completely crossing the nail of all fingers at the same relative distance from the base. Both arsenites and arsenates undergo enzymatic methylation in cells of various organisms, e.g. bacteria, rat, human and fish among others, producing monomethylarsonic (MMA) and dimethylarsinic acids (DMA). These reactions are catalyzed by methyltransferases that use S-adenosyl-methionine (SAM) as cofactor (Zhao et al., 1997) and are important because methylated arsenicals are more rapidly excreted in urine than inorganic arsenicals. The rate of methylation varies among species, and large inter-individual variations are observed in humans (Vahter, 1999; 2002; Zakharyan et al., 1995, 1996). Since toxicity of arsenicals is dictated by their rate of clearance from the body (Klaassen, 1996), methylation was considered for many years to be a biotransformation and detoxification mechanism (Buchet et al., 1981; Moore et al., 1997; Yamauchi and Fowler, 1994). However, recent evidence has shown that methylated trivalent arsenic species are more toxic than inorganic arsenic (e.g. Ahmad et al., 2000; Mass et al., 2001; Petrick et al., 2000; see review by Thomas et al., 2001), thus methylation may not solely be a detoxification mechanism for this metalloid but could instead be considered a pathway for its activation (Hughes, 2002). Indeed, more arsenic effects are observed in human beings that present a higher methylation rate than in those that excrete less methylated species and more inorganic arsenic. This later finding agrees with a higher toxicity of the methylated arsenic compounds (Chung et al., 2002).

2.2.4 Toxicity of Arsenic

While arsenic occurs in both organic and inorganic compounds, the two types behave differently in the human body. Organic arsenic compounds are not metabolized easily and, when ingested, are excreted unchanged (ASTDR 2007). Inorganic compounds of arsenic are metabolized, forming monomethylarsonic acid and dimethylarsinic acid (ASTDR 2007).

Human exposure to inorganic arsenic can occur in several different ways: Dermal or skin exposure, inhalation, and ingestion. Inorganic arsenic is classified as a Class A Carcinogen, or Human Carcinogen, by the U.S. Environmental Protection Agency when exposure is ingestion or inhalation (U.S. Environmental Protection Agency 1998).

Dermal/skin exposure: may result in skin irritation with local redness and/or swelling but has no permanent toxic effects (ASTDR 2007).

Inhalation: exposure of this kind can be occupational, such as refinery workers and farmers. Exposure of greater than .75 mg/m3 is associated with a greater risk of lung cancer (World Health Organization, 2001; ASTDR 2007).

Ingestion: Large doses (from 50 to 300 mg) of ingested arsenic are fatal (ASTDR 2000). Smaller doses can come from food and/or drinking water and cause symptoms such as stomachache, nausea, vomiting, and diarrhea as well as decreased production of red and white blood cells, which may cause fatigue, abnormal heart rhythm, blood-vessel damage resulting in bruising, and impaired nerve function causing a pin and needles sensation in your hands and feet (ASTDR 2007). Long term exposure from 5 to 15 years can lead to various cancers including bladder, liver, lung and skin. Other skin diseases may develop including patches of roughened skin on the palms and soles, which can be a hallmark of chronic exposure (ASTDR 2007; World Health Organization 2001).

Arsenic is mainly excreted from the body through urine within 1 to 2 days. (ASTDR 2007) Indicators of exposure to arsenic can be measured in urine but can also occur in hair, nails and blood.

2.3.0 Antioxidants

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances (Sies, 1997).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions

that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. For many years chemists have known that free radicals cause oxidation which can be controlled or prevented by a range of antioxidants substances (Bjelakovic et al., 2007). It is vital that lubrication oils should remain stable and liquid should not dry up like paints. For this reason, such oil usually has small quantities of antioxidants such as phenol or amine derivatives, added to them. Although plastics are often formed by free radical action, they can also be broken down by the same process, so they too, require protection by antioxidants like phenols or naphthol. Low density polythene is also of protected by carbon black which absorbs the ultraviolet light which causes radical production (Sies, 1997).

2.4.0 Catalase (EC 1.11.1.6)

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert

millions of molecules of hydrogen peroxide to water and oxygen each second (Bartoszek and Sulkowski, 2006).

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon et al., 2007). It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7 (Maehly and Chance, 1954), and has a fairly broad maximum (the rate of reaction does not change appreciably at pHs between 6.8 and 7.5) (Aebi, 1984). The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species (Toner et al., 2000).

2.4.1 Molecular Mechanism of Catalase

While the complete mechanism of catalase is not currently known (Boon et al., 2007), the reaction is believed to occur in two stages:

$H_2O_2 + Fe^{3+}\text{-}E \rightarrow H_2O + O{=}Fe^{4+}\text{-}E^{\cdot+}$

$H_2O_2 + O{=}Fe^{3+}\text{-}E^{\cdot+} \rightarrow H_2O + Fe^{3+}\text{-}E + O_2$

Here Fe^{+}-E represents the iron center of the heme group attached to the enzyme. $Fe^{4+}\text{-}E^{\cdot+}$ is a mesomeric form of Fe^{5+}-E, meaning the iron is not completely oxidized to +5, but receives some "supporting electrons" from the heme ligand. This heme has to be drawn then as a radical cation (.+).

As hydrogen peroxide enters the active site, it interacts with the amino acids Asn147 (asparagine at position 147) and His74, causing a proton (hydrogen ion) to transfer between the oxygen atoms. The free oxygen atom coordinates, freeing the newly formed water molecule and Fe^{4+}=O. Fe4+=O reacts with a second hydrogen peroxide molecule to reform Fe^{3+}-E and produce water and oxygen (Boon et al., 2007). The reactivity of the iron center may be improved by the presence of the phenolate ligand of Tyr357 in the fifth iron ligand, which can assist in the oxidation of the Fe^{3+} to Fe^{4+}. The efficiency of the reaction may also be improved by the interactions of His74 and Asn147 with reaction intermediates (Boon et al., 2007). In general, the rate of the reaction can be determined by the Michaelis-Menten equation (Maass, 1998).

Catalase can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

The exact mechanism of this reaction is not known.

2.4.2 Cellular Role of Catalase

Hydrogen peroxide is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules (Gaetani et al., 1996).

The true biological significance of catalase is not always straightforward to assess: Mice genetically engineered to lack catalase are phenotypically normal, indicating this enzyme is dispensable in animals under some conditions (Ho et al., 2004). A catalase deficiency may increase the likelihood of developing type 2 diabetes (László et al., 2001; László, 2008). Some humans have very low levels of catalase (acatalasia), yet show few ill effects. The predominant scavengers of H2O2 in normal mammalian cells are likely peroxiredoxins rather than catalase.

Catalase is usually located in a cellular, bipolar environment organelle called the peroxisome (Alberts et al., 2002). Peroxisomes in plant cells are involved in photorespiration (the use of oxygen and production of carbon dioxide) and symbiotic nitrogen fixation (the breaking apart of diatomic nitrogen (N2) to reactive nitrogen atoms). Hydrogen peroxide is used as a potent antimicrobial agent when cells are infected with a pathogen. Catalase-positive pathogens, such as Mycobacterium tuberculosis, Legionella pneumophila, and Campylobacter jejuni, make catalase to deactivate the peroxide radicals, thus allowing them to survive unharmed within the host (Srinivasa et al., 2003).

2.5.0 Superoxide Dismutase (EC 1.15.1.1)

Superoxide dismutase (SOD), is a metalloenzyme whose active center is occupied by copper and zinc, sometimes manganese and iron (Beyer *et al.,* 1991).

The enzyme superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD is endogenously produced intracellular enzyme present in essentially every cell in the body. There are at least three forms of superoxide dismutase in nature. Human erythrocytes contain an SOD enzyme with divalent copper and divalent zinc. Chicken liver mitochondria and E. coli contain a form with trivalent manganese. E. coli also contain a form of the enzyme with trivalent iron. The Cu-Zn enzyme is dimer of molecular weight 32,500. The two subunits are joined by a disulfide bond.

Cellular SOD is actually represented by a group of metalloenzymes with various prosthetic groups. The prevalent enzyme is the cupro-zinc (CuZn) SOD, which is a stable dimeric protein (Cass, 1985).

Superoxide dismutase is formed when reduced flavins present, for example, in xanthine oxidase, are reoxidized univalently by molecular oxygen.

$$\text{Enz-flavin-}H_2 + O_2 \longrightarrow \text{Enz-flavin-H} + O_2^- + H^+$$

Superoxide can reduce oxidized cytochrome c or be removed by superoxide dismutase.

$$O_2^- + \text{cyt c } (Fe^{3+}) \longrightarrow O_2 + \text{cyt c } (Fe_{2+})$$

$$O_2^- + O_2 + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

2.5.1 Role of Superoxide Dismutase in Health

Superoxide dismutase plays an extremely important role in the protection of cells against oxidative damage. The two major forms of superoxide dismutase in humans are the mitochondrial manganese SOD and the cytosolic copper/zinc SOD. A copper/zinc SOD, isolated from beef liver, has been used intra-articularly for degenerative joint disorders as an anti-inflammatory agent. Superoxide dismutase is also marketed as a nutritional supplement (Beyer et al., 1991).

Superoxide dismutase is also an effective defence weapon and Mycobacteria and Nocadia have SOD which enables them to resist the injection of superoxide by phagocytes. When these organisms cause serious disease, it takes the body a very long time to win, and depending on the strength of the patient, the bacteria may win. Although the enzyme isn't especially fast relative to the spontaneous dismutation of superoxide, the ability of the enzyme to provide some protection to organisms is shown by the existence of a motor neuron disease in individuals who have point mutations in SOD and by the finding that the absence of superoxide dismutase may lead to a form of anaemia (Bowler *et al.*, 1992; Nicholls *et al*., 2000). It is said that SOD protects the lens of the eyes by guiding against free radical damage.

2.6.0 Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogens. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination (Guéraud, *et al*., 2010).

2.6.1 Mechanism of Lipid Peroxidation

1. Initiation

Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH• and HO2, which combines with a hydrogen atom to make water and a fatty acid radical.

2. Propagation

The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way.

3. Termination

When a radical reacts with a non-radical, it always produces another radical, which is why the process is called a "chain reaction mechanism". The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals. Living organisms have different molecules that speed up termination by catching free radicals and, therefore, protecting the cell membrane. One important such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

2.6.2 Biological Roles of Lipid Peroxidation Products

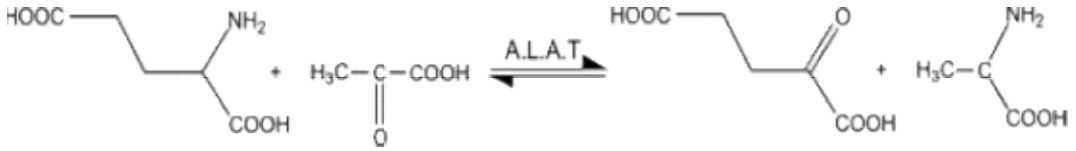
It has been known that lipid peroxidation gives complex products including hydroperoxides, cleavage products such as aldehydes, and polymeric materials, and that these products exert cytotoxic and genotoxic effects (Esterbauer, 1993). Lipid peroxidation products and modified proteins have been found in human atherosclerotic lesions, although their pathological significance, such as cause or consequence, has not yet been fully elucidated. More recently, the role of lipid peroxidation products as signaling messengers has received a great deal of attention (Poli, et al., 2004). For example, 9- and 13-HODE have been shown to act as activators and ligand of PPARc, leading to the induction of CD36 scavenger receptors and foam cell formation (Ostrea *et al*., 2008). Oxysterols are involved in the regulation of gene expression, and cholesterol metabolism and homeostasis (Repa and Mangelsdorf, 2002). Furthermore, it has been found that cyclopentenone prostaglandins, 15-deoxy-D12,14-prostaglandin J2 in particular, induce phase II detoxification enzymes (Kawamoto, et al., 2000), and exert a complex array of neurodegenerative, neuroprotective, and anti-inflammatory effects (Gueŕaud *et al*., 2010). Cells exhibit a broad spectrum of responses to oxidative stress, depending on the stress type and level encountered. Oxidative stress exceeding the antioxidant capacity level may induce oxidative damage, but low-level stress may enhance the defense capacity. Such an adaptive response has been observed in several instances, particularly in low-dose irradiation (Marnett, 1999). It was reported that the pretreatment of human umbilical vein endothelial cells with 15d-PGJ2 protected the cells from subsequent 4-HNE-induced apoptosis (Levonen et al., 2001). It was recently found that a sublethal level of 4-HNE exerted a cyto-protective effect primarily through the induction of thioredoxin reductase 1 against subsequent oxidative stress.

2.7.0 Aminotransferases

Aminotransferases, also called transaminases are present in most of the tissues of the body. They catalyze the interconversions of the amino acids and 2-oxacids by transfer of amino groups. Transaminases are specific for the amino acid from which the amino group has to be transferred to a keto acid. 2-oxoglutarate and glutamate couple serves as one amino group acceptor and donor pair in all amino transfer reactions (Pratt and Kaplan, 2000).

2.7.1 Alanine Aminotransferase (EC 2.6.1.2)

Alanine aminotransferase (ALT), also known as glutamate pyruvate transaminase (GPT), is a pyridoxal enzyme which belongs to the class-I pyridoxal-phosphate-dependent aminotransferase family, Alanine aminotransferase subfamily. Alanine aminotransferase / Gpt1 / ALT catalyses the reversible interconversion of L-alanine and 2-oxoglutalate to pyruvate and L-glutamate, and plays a key role in the intermediary metabolism of glucose and amino acids (Ghouri *et al*., 2010).



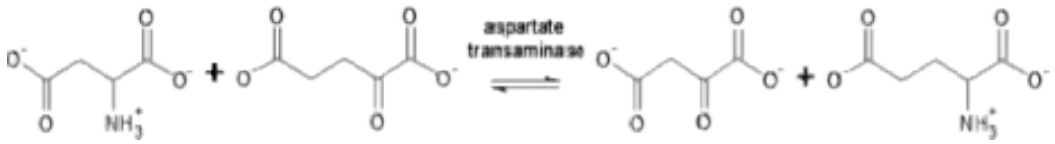
Alanine aminotransferase / Gpt1 / ALT is expressed in Liver, kidney, heart, and skeletal muscles and it expresses at moderate levels in the adipose tissue. As a key enzyme for gluconeogenesis, Alanine aminotransferase is a widely-used serum marker for liver injury (Ghouri *et al*., 2010). Two ALT isoenzymes have been identified, ALT1 and ALT2 (GPT1 and GPT2), which are encoded by separate genes and share significant sequence homology, but differ in their expression patterns. GPT1/Alanine aminotransferase is widely distributed and mainly expressed in intestine, liver, fat tissues, colon, muscle, and heart, in the order of high to low expression level (Goessling *et al*., 2008; Schindhelm *et al*., 2006). Serum activity levels of this enzyme are routinely used as a biomarker of liver injury caused by drug toxicity, infection, alcohol, and steatosis (Ghouri *et al*., 2010; Wang *et al*., 2012).

2.7.2 Aspartate Aminotransferase (EC 2.6.1.1)

Aspartate aminotransferase (AspAT/ASAT/AAT) or serum glutamic oxaloacetic transaminase (SGOT) is a pyridoxal phosphate (PLP)-dependent transaminase enzyme (EC 2.6.1.1). Aspartate aminotransferase catalyzes the reversible transfer of an α-amino group

between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism (Hayashi *et al.*, 1990). AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health (Goessling *et al*., 2008; Schindhelm *et al*., 2006).

Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate.



As a prototypical transaminase, AST relies on PLP as a cofactor to transfer the amino group from aspartate or glutamate to the corresponding ketoacid. In the process, the cofactor shuttles between PLP and the pyridoxamine phosphate (PMP) form (Kirsch *et al*., 1984). The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. In amino acid degradation, following the conversion of α-ketoglutarate to glutamate, glutamate subsequently undergoes oxidative deamination to form ammonium ions, which are excreted as urea. In the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the citric acid cycle (Berg *et al*., 2006).

2.7.3 Mechanism Of Aspartate Amino Transferase

Aspartate transaminase, as with all transaminases, operates via dual substrate recognition; that is, it is able to recognize and selectively bind two amino acids (Asp and Glu) with different side-chains (Hirotsu *et al*., 2005). In either case, the transaminase reaction consists of two similar half-reactions that constitute what is referred to as a ping-pong mechanism. In the first half-reaction, amino acid 1 (e.g., L-Asp) reacts with the enzyme-PLP complex to generate ketoacid 1 (oxaloacetate) and the modified enzyme-PMP. In the second half-reaction, ketoacid 2 (α-ketoglutarate) reacts with enzyme-PMP to produce amino acid 2 (L-Glu), regenerating the original enzyme-PLP in the process. Formation of a racemic product (D-Glu) is very rare (Kochhar and Christen, 1992).

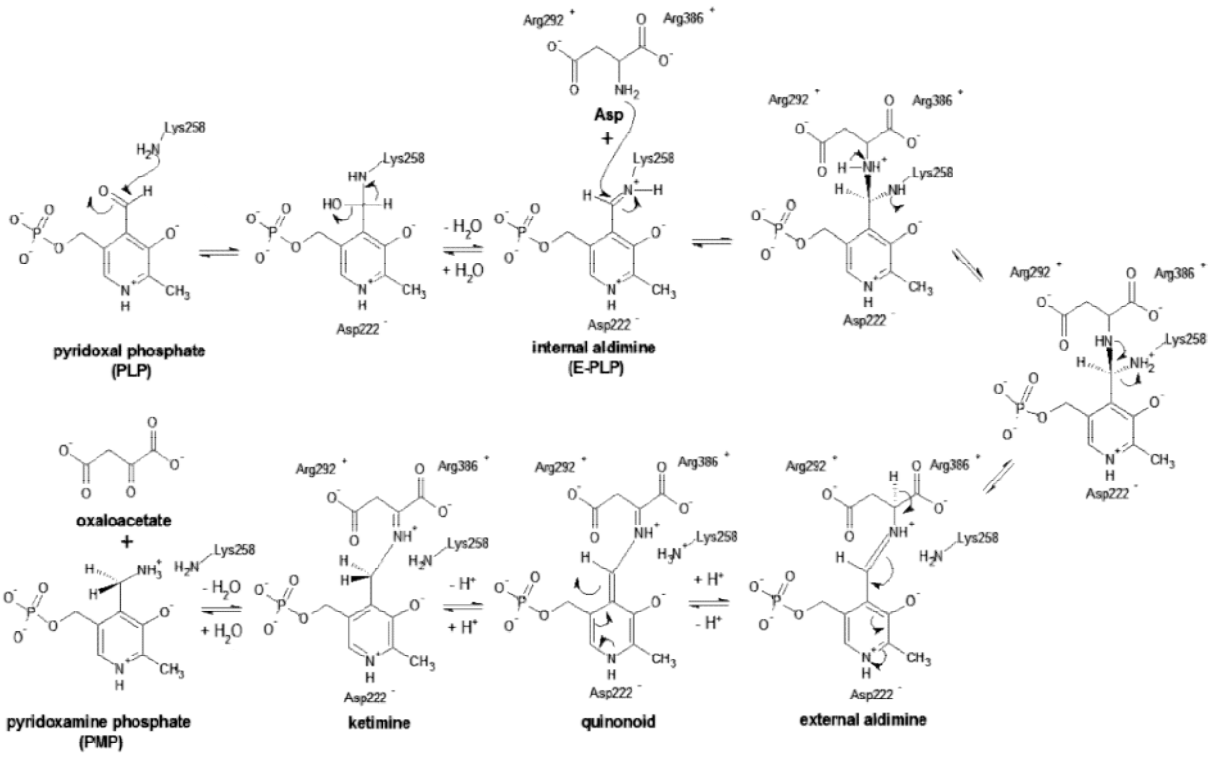


Fig 2.3: Reaction Mechanism for Aspartate Amino transferase (Kochhar and Christen, 1992)

2.7.4 Clinical Significance of Aminotransferases

The activities of both AST and ALT are high in tissues especially liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood. Some increases in the activities of both the enzymes are seen after alcohol intake (Goessling *et al*., 2008; Schindhelm *et al*., 2006).

Determinations of activities of AST and ALT in serum in patients with liver diseases like viral hepatitis and other forms of liver diseases with necrosis, give high values even before the appearance of clinical signs and symptoms like jaundice (Gaze, 2007). Activity levels of 20 to 50 fold higher than normal are frequently seen in liver cells damage but it may reach as high as 100 times in severe damage to cells. In myocardial infarction high activity of AST is seen in serum. ALT activity is within normal range or slightly increased in uncomplicated myocardial infarction. Rise in AST is seen within 6 to 8 hours of the onset of chest pain, highest level at 18 to 24 hours and returns to pre-infarction levels by 4th to 5th day (Walker and Lorimer, 2004). There are other superior markers available for myocardial infarction as AST lacks the tissue specific characteristics, as its activity may also increased in

diseases of other tissues like liver and skeletal muscles (Walker and Lorimer, 2004; Pratt and Kaplan, 2000). In other conditions like pulmonary emboli, acute pancreatitis, hemolytic disease and gangrene the activity of AST is found to be 2 to 5 times higher than the normal activity (Giannini *et al*., 1999; Nsiah *et al.*, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Materials

3.1.1 Apparatus/Instruments

1. Centrifuge - Remi's motor Ltd, India
2. Water bath (DK 420) - Techmel and Techmel, USA
3. Spectrophotometer(UV-752) - Gnang Zhou guo Leng electronic machines, China
4. Electronic weighing balance - Metlar, China.
5. Colorimeter - Biomed care, Delhi, India
6. pH meter - Shanghai Weiye Instruments, China
7. Dessicator

3.1.2 Chemicals and Manufacturers

All chemicals used were of laboratory grade.

1. Chloroform - May and baker, England.
2. Sodium carbonate - Avondale laboratories, England.
3. Sodium hydrogen carbonate - BDH chemicals, England.
4. Sodium hydroxide - Burgoyne burbidges, India.
5. Epinephrine - BDH, Poole, England.
6. Hydrochloric acid - May and Baker, England.
7. Hydrogen peroxide - BDH, Poole, England.
8. Disodium hydrogen phosphate - Avondale laboratories, England.
9. Sodium dihydrogen phosphate - BDH, Poole, England.
10. Glacial acetic acid - May and Baker, England.
11. Potassium hydroxide - Avondale laboratories, England.
12. Potassium dichromate - BDH, Poole, England.
13. Cadmium Chloride - E. Merck, Darmstadt.
14. Arsenic trioxide - Rajnish Inc., New Delhi, India.
15. AST assay kit - Randox laboratory Ltd, UK.
16. ALT assay kit - Randox laboratory Ltd, UK.

3.1.3 FISHES

For the purpose of compounding the diets for the experimental animals, catfishes were purchased from Delta State Government owned fish farm, Obiaruku, Delta State. The fishes were divided into four groups and left to acclimatize for two (2) weeks.

- **Group A (Control)** - Fishes in this group were housed in fresh water. This served as normal control group.
- **Group B (cadmium)** - The water was contaminated with cadmium (0.4mg/100ml). The water was changed and re-contaminated every 24 hours for four weeks.
- Group C (Arsenic) - The water was contaminated with arsenic (0.4mg/100ml). The water was changed and re-contaminated every 24 hours for four weeks.
- **Group D (cadmium + arsenic)** - The water was contaminated with cadmium and arsenic (0.4mg/100ml of each contaminant). The water was changed and re-contaminated every 24 hours for four weeks.

  All the fishes received normal feed for the duration after which they were killed, dried in an oven and used as protein source for both control and test diets.

3.1.3 Preparation of Diet

The diet was prepared as follows using the heavy metal exposed fishes as protein source for the test diets and non-metal exposed fishes as source of protein for the control diet.

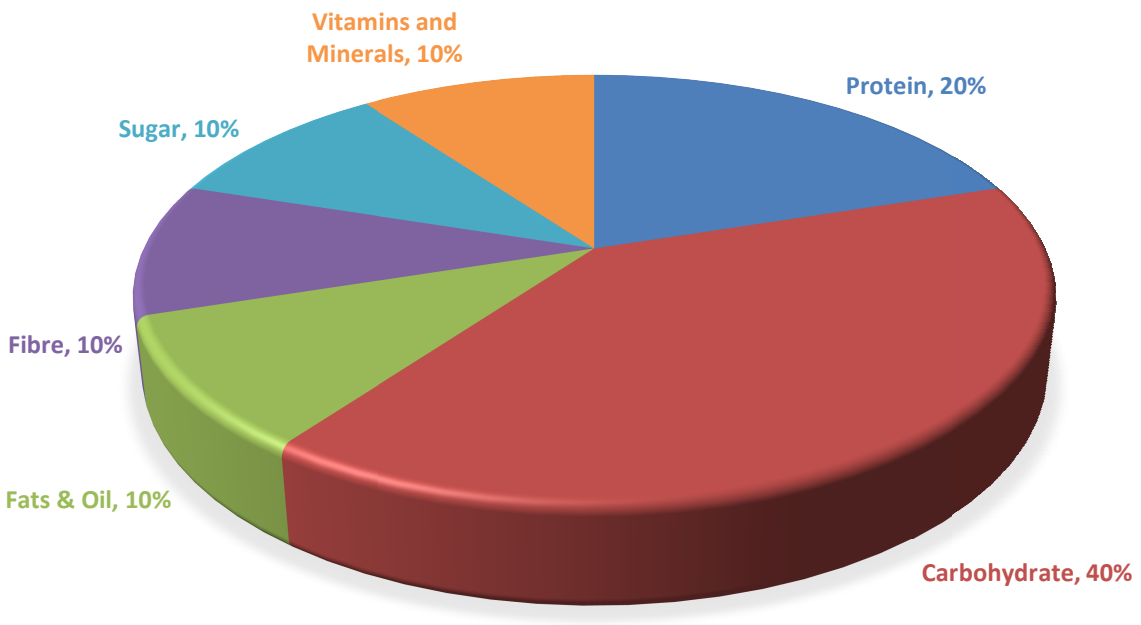


Fig 3.1 Diet composition.

1. **Protein:** African catfish prepared as described above served as source of protein for both control and test diets and it made up 20% of the total control and test diets.
2. **Carbohydrate:** Corn starch served as the source of carbohydrate and it made up 40% of the total diet.
3. **Fats & Oil:** Vegetable oil served as the source of fats & oil and it made up 10% of the total diet.
4. **Fibre:** Laboratory cellulose served as the source of fibre and it made up 10% of the total diet.
5. **Sugar:** Granulated refined sugar served as the source of sugar and it made up 10% of the total diet.
6. **Vitamins and Minerals:** Vitamins and mineral mix (manufactured by Hebei Vsyong Animal Phamaceutical Co. Ltd, China) made up 10% of the total diet. The minerals and vitamins were in the following proportion per 1kg of the mix.

Vitamin A	5,000,000 I.U
Vitamin E	15,00mg
Vitamin B2	25,00mg
Vitamin B6	1000mg
Vitamin D3	500,000 I.U
Vitamin B1	1000mg
Vitamin C	2000mg
Vitamin K3	250mg
Pantothenic acid	2000mg
Carnitine HCl	1500mg
Folic acid	50mg
Potassium Chloride	62.5g
Nicotinic acid	3000mg
Methionine	7500mg
Sodium Chloride	62.5g
Calcium Chloride	62.5g
Anhydrous Glucosese	Q.S

3.1.4 Experimental Animals

Twenty-eight (28) adult male albino rats of Wistar strain weighing between 150-200g were procured from the animal house, College of Health Science, Delta State University, Abraka, Nigeria. The animals were housed in standard animal cage and maintained under controlled environmental condition with a 12 h dark: light cycle.

3.2.0 Methods

3.2.1 Experimental Design

The experimental design mimicked a model food-chain of fish to rat by feeding various groups of rats with their respective diets.

Twenty-eight (28) male albino rats of Wistar strain were divided into four (4) groups with seven (7) rats in each group. The treatment for each of the group are as follows;

- **Group A (Control)** - Animals in this group were fed with normal diet containing non-metal exposed fish as a source of protein for twelve (12) weeks. This served as normal control group.
- **Group B (cadmium)** - Animals in this group were fed with diet containing cadmium contaminated fish for twelve (12) weeks.

- **Group C (arsenic)** - Animals in this group were fed with diet containing arsenic contaminated fish for twelve (12) weeks.
- **Group D (cadmium + arsenic)** - Animals in this group were fed with diet containing cadmium + arsenic contaminated fish for twelve (12) weeks.

  Animals in all the groups were allowed free access to drinking water throughout the period of the study.

3.2.2 Collection of Tissues

After the appropriate treatment periods, the animals were weighed and sacrificed under chloroform anesthesia. Blood was obtained from the heart of each rat using hypodermic syringe and needle. The liver, kidney, brain, testis, prostrate and heart were dissected out, washed in normal saline, blotted individually on ash free filter paper, patted dry and weighed. The weighed tissues were stored in separate containers, labeled and immediately transferred to ice packs awaiting homogenization. Plasma was obtained after centrifugation at 3000g for 10 minutes.

3.2.3 Preparation of Samples

Ten percent homogenates of the organs were prepared in normal saline (0.9% NaCl) using mortar and pestle under cold conditions. The homogenates were centrifuged at 5000g for 10 minutes and the supernatants obtained were subsequently stored in a refrigerator preparatory for biochemical analysis.

3.3.0 Biochemical Assays

3.3.1 Assay for Alanine Aminotransferase Activity

The assay for Alanine Aminotransferase in the plasma and tissue was carried out by the method described by Reitman and Frankel (1957) using a kit by randox laboratories, UK.

Principle

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{GPT}} \text{L-glutamate} + \text{pyruvate}$$

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Reagent Composition

	Contents	Initial Concentration of Solutions
R1	Buffer	
	Phosphate Buffer	100 mmol/l, pH 7.4
	L-alanine	200 mmol/l
	α-oxoglutarate	2.0 mmol/l
R2	2,4-dinitrophenylhydrazine	2.0 mmol/l

Procedure

The assay was carried out by adding the following into labelled test tubes.

	Reagent Blank	**Sample**
Sample	---	0.1 ml
Solution R1	0.5 ml	0.5 ml
Distilled water	0.1 ml	---

The solutions were mixed and incubated for 30 minutes, at 37°C after which to each test tube, 0.5ml of solution R2 (2,4-dinitrophenylhydrazine) was added. The solutions were mixed and allowed to stand for 20 minutes, at 25°C. 5ml of 0.4M NaOH was added to each solution, mixed and allowed to stand for 5 minutes after which absorbance was read at 520nm.

The activity of Alanine aminotransferase was extrapolated from the standard curve which was plotted and was expressed in unit/ul

3.3.2 Assay for Aspartate Aminotransferase Activity

The assay for plasma and tissue Aspartate Aminotransferase was carried out by the method described by Reitman and Frankel (1957) using Randox Laboratories kit.

Principle

α-oxoglutarate + L-aspartate $\xrightarrow{\text{GPT}}$ L-glu + oxaloacetate

Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Reagent Composition

	Contents	Initial Concentration of Solutions
R1	Buffer	
	Phosphate Buffer	100 mmol/l, pH 7.4
	L- aspartate	100 mmol/l
	α-oxoglutarate	2 mmol/l
R2	2,4-dinitrophenylhydrazine	2 mmol/l

Procedure

The assay was carried out by adding the following into labelled test tubes.

	Reagent Blank	**Sample**
Sample	---	0.1 ml
Solution R1	0.5 ml	0.5 ml
Distilled water	0.1 ml	---

The solutions were mixed and incubated for 30 minutes, at 37°C after which to each test tube 0.5ml of solution R2 (2,4-dinitrophenylhydrazine) was added. The solutions were mixed and allowed to stand for 20 minutes, at 25°C. 5ml of 0.4M NaOH was added to each solution, mixed and allowed to stand for 5 minutes after which absorbance was read at 520nm.

The activity of Aspartate Aminotransferase was extrapolated from the standard curve plotted and expressed in units/ml.

3.3.3 Assay for Superoxide Dismutase Activity

The assay for superoxide dismutase (SOD) activity was done by the method of Misra and Fridovich (1972).

Principle

The assay for SOD is an indirect method which is based on the inhibitory effects of SOD in the initial rate of epinephrine autooxidation which is derived from the reaction proposed by Misra and Fridovich, (1972), for the base catalyzed autooxidation of epinephrine.

Procedure

The assay was carried out by adding 0.2ml of the supernatant to 2.5ml 0.05M carbonate buffer, pH 10.2. The reaction was started by the addition of 0.3ml freshly prepared 0.3mM epinephrine as the substrate to the buffer-supernatant mixture and was quickly mixed by inversion. The reference cuvette contained 2.5ml of the buffer, 0.3ml of the substrate and

0.2ml of the distilled water. The increase in absorbance at 480nm due to the adenochrome formed was monitored every 30 seconds for 150 seconds. 1 unit of SOD necessary to cause 50% inhibition of the oxidation of epinephrine to adenochrome during 1 minute.

Calculation

Percent inhibition =

$$100 - 100 \times \left\{ \frac{\text{Epinephrine oxidation in the presence of SOD}}{\text{Epinephrine oxidation in the absence of SOD}} \right\}$$

$$\text{Units/g wet tissue} = \frac{\%\ \text{inhibition} \times 1 \times D}{x' \times 50}$$

Where x' = g of tissue in the reaction mixture

$^{1}/_{50}$ = converts to 50% inhibition

D = Dilution factor

Where 1 unit of SOD activity = Amount of SOD giving 50% inhibition

3.3.4 Assay for Lipid Peroxidation

A breakdown product of lipid peroxidation, thiobarbituric acid reactive substance (TBARS) was measured in the plasma and tissue by the method of Gutteridge and Wilkins (1982).

Principle

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acid (PUFA) served as a convenient index for the determination of extent of peroxidation reaction. Malondialdehyde was identified with thiobarbituric acid to give a red species absorbance at 532nm.

Procedure

The sample (0.2ml) was added to 1ml glacial acetic acid followed by 1ml 1% TBA. The loosely stoppered tubes were immersed in boiling water bath for 15mins, allowed to cool and centrifuged at 800g for 15mins. The clear supernatant was carefully transferred into a cuvette and absorbance read at 532nm against reagent blank. A molar extinction coefficient of 1.56 x 10^5 M^{-1} was used according to the expression of Adam-Vizi and Seregi (1982).

Calculation

$$MDA\ (unit/g\ tissue) = \frac{\Delta A\ x\ V}{\Sigma 532\ x\ V_s\ x\ X^1}$$

Where,

ΔA	=	Change in absorbance (nm)
V	=	Total volume of reactants
X^1	=	Weight of tissue in reaction mixture
Σ	=	Molar absorbance index = 1.56 x 10^5 MCN^{-1}
V_s	=	Volume of sample in reaction mixture.

3.3.5 Assay for Catalase Actvity

Catalase activity was assayed in the plasma and tissue according to the method of Sinha (1972).

Principle

Catalase activity is estimated by measuring the breakdown of hydrogen peroxide in the reaction mixture using a colorimeter at the wavelength of 610nm

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Catalase breaks down H_2O_2 directly into water and oxygen. The decrease in H_2O_2 concentration can be monitored.

Procedure

The enzyme extract (0.5ml) was added to the reaction mixture containing 1ml of 0.05M phosphate buffer (pH 7.0), 0.5ml of 0.2M H_2O_2, 0.4ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2ml of acid reagent (dichromate/acetic acid mixture) which was prepared by missing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10mins and the absorbance was read at 610nm. Catalase activity was expressed in terms of moles of H_2O_2 consumed/min/mg protein.

Calculation

The disappearance of hydrogen peroxide may be described by the equation for first order kinetics

$$\text{Log}_{10}\, A = \log_{10} A_O - {}^{kt}/_{2.3}$$

$$\text{Therefore k} = \frac{\log_{10} A}{\log_{10} A_0} \; x \; \frac{2.3}{t}$$

Where,

A = absorbance of H_2O_2 at time t seconds

A_O = absorbance of H_2O_2 at time zero (0) seconds

K = rate constant

t = time in minutes.

3.4.0 Statistical Analysis

All data generated were subjected to statistical analysis. This was done using analysis of variance (ANOVA) and least significance difference (LSD) test.

CHAPTER FOUR

RESULT

4.1: Effect of Food Chain Mediated Exposure to Cadmium and Arsenic on Body and Organ Weight

The results of the analysis carried out are shown in Tables 1-4 below.

Table 4.1 presents the effect of food chain mediated exposure to cadmium and arsenic on body weight and organ to body weight ratio. The body weight gain of rats fed arsenic and cadmium contaminated diet was significantly decreased relative to control. Conversely, no significant difference was observed in the body weight gain of rats fed a combination of arsenic and cadmium contaminated diet as compared to control. The organ/body weight ratio for the liver, testis, heart and brain was not significantly different in all experimental groups. Similarly, no significant difference ($p > 0.05$) was observed in the kidney/body weight ratio of rats fed diet contaminated with cadmium and a combination of cadmium and arsenic relative to control. On the other hand, this parameter was significantly increased in rats fed arsenic via the food chain. Also a significant increase was observed ($p<0.05$) in the prostrate/body weight ratio of rats fed diet containing cadmium and a combination of arsenic and cadmium relative to control. This parameter was however decreased significantly ($p<0.05$) in rats fed arsenic through the food chain.

Table 4.1: Effect of food chain mediated exposure to cadmium and arsenic on body weight gain and organ to body weight Ratio

Parameter	Control	Arsenic	Cadmium	Arsenic + Cadmium
Body weight Gain (g)	77.50 ± 7.59^{a}	39.27 ± 11.65^{b}	37.62 ± 5.85^{c}	80.07 ± 5.39^{a}
Liver/Body Weight Ratio	0.0339 ± 0.0012^{a}	0.0350 ± 0.0005^{a}	0.0305 ± 0.0028^{a}	0.0339 ± 0.0033^{a}
Kidney/body Weight Ratio	0.00610 ± 0.00003^{a}	0.00882 ± 0.00042^{b}	0.00718 ± 0.00025^{a}	0.00770 ± 0.00119^{a}
Testis/body Weight Ratio	0.01230 ± 0.00160^{a}	0.01457 ± 0.00177^{a}	0.01333 ± 0.00085^{a}	0.01130 ± 0.00066^{a}
Heart/body Weight Ratio	0.00380 ± 0.00020^{a}	0.00473 ± 0.00023^{a}	0.00377 ± 0.00032^{a}	0.00461 ± 0.00060^{a}
Prostrate/Body Weight Ratio	0.00452 ± 0.00051^{a}	0.00318 ± 0.00095^{b}	0.00572 ± 0.00028^{c}	0.00602 ± 0.00078^{c}
Brain/Body Weight Ratio	0.00802 ± 0.00080^{a}	0.01093 ± 0.00237^{a}	0.00856 ± 0.00063^{a}	0.00844 ± 0.00080^{a}

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P < 0.05$ using analysis of variance (ANOVA).

4.2 Effect of Food Chain Mediated Exposure to Cadmium and Arsenic on Biochemical Parameters

Table 4.2 shows the effect of arsenic, cadmium and a combination of both metals on tissue and plasma superoxide dismutase activity in rats. The testis and brain superoxide dismutase activity of rats fed arsenic, cadmium and a combination of cadmium and arsenic contaminated diet was not significantly different ($p>0.05$) relative to control and to one another. Also, the heart and liver superoxide dismutase activity of rats offered arsenic and cadmium contaminated diet was not significantly different when compared to control. The prostrate superoxide dismutase activity of rats fed cadmium and a combination of cadmium and arsenic contaminated diet was not significantly different from control. Similarly, there was no significant difference in plasma superoxide dismutase activity of rats fed a combination of cadmium and arsenic contaminated diet relative to control. Conversely, there was significant decrease in plasma superoxide dismutase activity of rat fed separately with arsenic and cadmium when compared with control, and those offered cadmium and arsenic together. There was significant increase ($p<0.05$) in kidney superoxide dismutase activity of rats fed arsenic, cadmium and a combination of cadmium and arsenic contaminated diet when compared to control but no significant difference when compared to one another. Also there was significant increase in heart and liver superoxide dismutase activity of rats fed a combination of arsenic and cadmium when compared to control and arsenic and cadmium offered separately. Also there was a significant decrease in prostrate superoxide dismutase activity of rats fed arsenic contaminated diet when compared to control, cadmium and a combination of cadmium and arsenic groups.

Table 4.2: Effect of food-chain mediated Exposure to Cadmium and Arsenic on Tissue and Plasma Superoxide Dismutase Activity of Rats

SOD (units/g tissue Weight)	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	90.00 ± 16.12[a]	86.0 ± 3.46[a]	82.00 ± 15.7[a]	112.0 ± 7.48[b]
(% change)		(*-4.44%*)	(*-8.88%*)	(*24.44%*)
Kidney	57.00 ± 15.35[a]	78.00 ± 8.72[b]	88.00 ± 3.65[b]	74.00 ± 12.27[b]
(% change)		*(36.84%)*	*(54.39%)*	(29.82*%)*
Testis	88.00 ± 2.83[a]	95.00 ± 8.70[a]	105.0 ± 6.40[a]	109.00 ± 1.0[a]
(% change)		*(7.95%)*	*(19.32%)*	*(23.86%)*
Heart	82.00 ± 6.00[a]	94.00 ± 13.22[a]	86.00 ± 2.58[a]	154.00 ± 41.59[b]
(% change)		*(14.63%)*	*(4.88%)*	*(87.80%)*
Prostrate	101.00 ± 18.28[a]	86.00 ± 2.58[b]	116.0 ± 9.09[a]	103.00 ± 7.72[a]
(% change)		*(-14.87%)*	*(14.85%)*	*(1.98%)*
Brain	97.00 ± 7.72[a]	92.0 ± 6.53[a]	103.00 ± 3.79[a]	98.00 ± 6.00[a]
(% change)		*(-5.15%)*	*(6.2%)*	*(1.03%)*
Plasma	113.00 ± 16.12[a]	95.00 ± 7.19[b]	104.0 ± 4.32[b]	116.00 ± 18.83[a]
(% change)		*(-15.93%)*	*(-7.96%)*	*2.65%*

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$ using analysis of variance (ANOVA). Values expressed in bracket indicate percentage change relative to control.

Table 4.3 shows the effect of food-chain mediated exposure to cadmium and arsenic on tissue and plasma catalase activity of rats. The heart, kidney, brain, prostrate and plasma catalase activity of rats offered arsenic, cadmium and a combination of cadmium and arsenic contaminated diet was not significantly different ($p>0.05$) relative to control. Also the testis catalase activity of rats offered arsenic, cadmium and a combination of both metals showed no significant difference when compared to one another. Similarly, there was no significant difference in liver catalase activity of rats treated with a combination of cadmium and arsenic as compared to control, but the activity of the enzyme was significantly decreased in the cadmium and arsenic treated groups. There was also a significant decrease ($p<0.05$) in testis catalase activity of rats offered arsenic, cadmium and a combination of both metals in diet relative to control.

Table 4.3: Effect of Food-Chain Mediated Exposure to Cadmium and Arsenic on Tissue and Plasma Catalase Activity of Rats

CAT (units/ml Tissue Homogenate)	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	0.87 ± 0.11^{a}	0.60 ± 0.06^{b}	0.42 ± 0.04^{b}	0.76 ± 0.16^{a}
(% change)		(-31.03%)	(-51.92%)	(-12.64%)
Kidney	1.70 ± 0.28^{a}	1.32 ± 0.29^{a}	1.33 ± 0.09^{a}	1.34 ± 0.09^{a}
(%change)		(-22.35%)	(-21.76%)	(-21.18%)
Testis	2.03 ± 0.35^{a}	1.09 ± 0.15^{b}	0.99 ± 0.08^{b}	0.94 ± 0.22^{b}
(%change)		(-46.31%)	(-51.23%)	(-53.69%)
Heart	0.92 ± 0.33^{a}	0.68 ± 0.13^{a}	0.79 ± 0.19^{a}	1.16 ± 0.23^{a}
(%change)		(-26.09%)	(-14.13%)	(26.09%)
Prostrate	1.27 ± 0.44^{a}	1.75 ± 0.18^{a}	1.12 ± 0.21^{a}	1.57 ± 0.10^{a}
(%change)		(37.80%)	(-11.81%)	(23.62%)
Brain	1.35 ± 0.01^{a}	1.39 ± 0.04^{a}	1.57 ± 0.07^{a}	1.40 ± 0.11^{a}
(%change)		(2.96%)	(16.30%)	(3.57%)
Plasma	1.53 ± 0.21^{a}	1.59 ± 026^{a}	1.48 ± 0.09^{a}	1.24 ± 0.07^{a}
(%change)		(3.92%)	(-3.27%)	(-18.95%)

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$ using analysis of variance (ANOVA). Values expressed in bracket indicate percentage change relative to control.

Table 4.4 shows the effect of arsenic, cadmium and a combination of both metals on tissue and plasma malonedialdehyde levels in rats. The heart, kidney, prostrate, liver and plasma malonedialdehyde levels of rats fed arsenic contaminated diet was not significantly different ($p>0.05$) relative to control and to one another. Similarly, there was no significant difference in brain malonedialdehyde level of rats offered cadmium and a combination of cadmium and arsenic contaminated diet when compared to control and to each other. However, there was a significant increase in testis malonedialdehyde level of rats offered cadmium, arsenic and a combination of these metals in diet relative to control. Similarly, there was a significant increase ($p<0.05$) in brain malonedialdehyde level of rats offered arsenic contaminated diet when compared to control, cadmium, and a combination of cadmium and arsenic groups.

Table 4.4: Effect of food-chain mediated exposure to cadmium and arsenic on the level of tissue and plasma Malonedialdehyde in rats (µmolg^{-1} tissue) or µmolml^{-1} for Plasma

MDA	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	1.24 ± 0.21^{a}	1.11 ± 0.27^{a}	1.56 ± 0.39^{a}	1.10 ± 0.15^{a}
(%change)		(-10.48%)	(25.81%)	(-11.29%)
Kidney	1.59 ± 0.26^{a}	1.41 ± 0.06^{a}	1.31 ± 0.28^{a}	1.06 ± 0.11^{a}
(%change)		(-11.32%)	(-17.61%)	(-33.33%)
Testis	0.83 ± 0.53^{a}	1.50 ± 0.15^{b}	1.91 ± 0.90^{c}	2.14 ± 0.64^{c}
(%change)		(80.72%)	(83.00%)	(157.83%)
Heart	0.84 ± 0.38^{a}	1.19 ± 0.06^{a}	1.31 ± 0.28a	1.06 ± 0.11^{a}
(%change)		(41.67%)	(55.95%)	(26.19)
Prostrate	0.62 ± 0.19^{a}	0.54 ± 0.19^{a}	0.50 ± 0.11^{a}	0.43 ± 0.20^{a}
(%change)		(-12.90%)	(-19.35%)	(-30.65%)
Brain	1.29 ± 0.17^{a}	2.17 ± 0.38^{b}	1.88 ± 0.61^{a}	1.48 ± 0.30^{a}
(%change)		(68.22%)	(45.74%)	(14.73%)
Plasma	0.53 ± 0.05^{a}	0.72 ± 0.06^{a}	0.74 ± 0.14^{a}	0.67 ± 0.06^{a}
(%change)		(35.85%)	(39.62%)	(26.42%)

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P < 0.05$), values not sharing a common superscript in same row differs at $P < 0.05$ using analysis of variance (ANOVA). Values expressed in bracket indicate percentage change relative to control.

Table 4.5 shows the effect of arsenic, cadmium and a combination of both metals on tissues and plasma aspartate aminotransferase activity of rats. There was no significant difference ($p>0.05$) in plasma AST activity of rats fed arsenic contaminated diet compared with control. Also, no significant difference occurred in heart AST activity of rats fed a combination of cadmium and arsenic contaminated diet relative to control. Similarly there was no significance difference in kidney AST activity of rats fed cadmium contaminated diet when compared to control. However, there was significant decrease and increase ($p<0.05$) in plasma AST activity of rats fed with cadmium and a combination of cadmium and arsenic contaminated diet respectively when compared to control and arsenic groups. Also there was significant decrease in heart AST activity of rats fed with arsenic and cadmium contaminated diet when compared to the control and those fed a combination of both metals. Similarly, there was significant decrease in liver AST activity of rats fed with arsenic, cadmium and a combination of cadmium and arsenic contaminated diet relative to control. Also, kidney AST activity of rats fed with arsenic and a combination of cadmium and arsenic contaminated diet was significantly decreased when compared to control and cadmium groups.

Table 4.5: Effect of food-chain mediated exposure to cadmium and arsenic on tissue and plasma aspartate aminotransferase activity of rats

AST (U/L)	Control	Arsenic	Cadmium	Arsenic+Cadmium
Liver	437.5 ± 115.64^{a}	1155 ± 231.28^{b}	1247 ± 82.50^{b}	962.5 ± 96.38^{b}
(%change)		(164%)	(185.03%)	(120.14%)
Kidney	380 ± 34.64^{a}	170 ± 47.26^{b}	310 ± 10.00^{a}	210 ± 30.00^{c}
(%change)		(-55.26%)	(-18.42%)	(-44.74%)
Heart	595 ± 35.00^{a}	262 ± 77.60^{b}	210 ± 40.41^{b}	420 ± 70.00^{a}
(%change)		(-55.97%)	(-64.71%)	(-29.41%)
Plasma	38.75 ± 6.01^{a}	38.50 ± 12.94^{a}	12.25 ± 3.35^{c}	55.50 ± 10.20^{b}
(%change)		(0.65%)	(-68.39%)	(43.23)

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$ using analysis of variance (ANOVA). Values expressed in bracket indicate percentage change relative to control.

Table 4.6 shows the effect of arsenic cadmium and a combination of both metals on tissue and plasma alanine aminotransferase activity of rats.

There was no significant difference in ($p>0.05$) heart ALT activity of rats fed arsenic, cadmium and a combination of cadmium and arsenic contaminated diet relative to control. Also there was no significant difference in kidney ALT of rats fed a combination of cadmium and arsenic contaminated diet when compared with the control. Similarly there was no significant difference in plasma ALT activity of rats fed arsenic contaminated diet relative to control.

However, there was significant increase ($p<0.05$) in liver ALT activity of rats fed arsenic, cadmium and a combination of cadmium and arsenic contaminated diet when compared to control. Conversely, kidney ALT activity of rats fed arsenic and cadmium contaminated diet was significantly decreased in relative to control and a combination of cadmium and arsenic groups. On the other hand the plasma ALT activity of rats fed cadmium and a combination of cadmium and arsenic contaminated diet was significantly increased relative to control.

Table 4.6: Effect of Food-chain Mediated Exposure to Cadmium and Arsenic on Tissue and Plasma Alanine Aminotransferase Activity of Rats

Parameter	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	945 ± 20.20^{a}	2555 ± 132.50^{b}	3097 ± 128.99^{c}	3185 ± 35.00^{c}
(%change)		(170.39%)	(227.72%)	(237.04%)
Kidney	110.00 ± 70.00^{a}	40.00 ± 0.00^{b}	40.00 ± 0.00^{b}	130.00 ± 10.00^{a}
(%change)		(-63.64%)	(-63.64%)	(18.18%)
Heart	1067.50 ± 100.53^{a}	1102 ± 72.15^{a}	910 ± 28.58^{a}	1085 ± 60.62^{a}
(%change)		(3.23%)	(-14.75%)	(1.64%)
Plasma	28.00 ± 4.95^{a}	31.50 ± 6.06^{a}	45.50 ± 2.02^{b}	45.50 ± 8.33^{b}
(%change)		(12.50%)	(62.50%)	(62.50%)

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$ using analysis of variance (ANOVA). Values expressed in bracket indicate percentage change relative to control.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1.0 Discussion

Cadmium and arsenic are two heavy metals that attracted the interest of researchers due to their hazardous nature. The common oral routes of administration of these metals in many studies are by addition to food or water but not through the food-chain, which is the natural routes of entry.

The purpose of the present study was to evaluate the effect of cadmium and arsenic administered via the food-chain on some biochemical parameters of rats. The decreased body weight gain of rats separately administered cadmium and arsenic (Table 4.1) tainted food is an indication of toxicity of these metals. Similarly, the alteration in prostrate/body weight ratio of rats administered cadmium arsenic or their combination is also an indication of toxicity. Changes in the body weight gain and organ/body weight ratio are important indices for toxicity of chemicals (Thimbrell, 1991). Studies by Grosiski and Kowalski (2002) indicate that cadmium, mercury and lead combination administered in drinking water decreased markedly body weight gain of rats but no visible difference were observed in organ/body weight ratios.

The lack of significant change in the level of SOD in the liver, brain and teastis of rats offered cadmium, arsenic and their combination (Table 4.2) is an indication of a lack of oxidative stress in these organs. Exposure to heavy metals has been linked to oxidative stress occasioned by increased production of free radicals (Flora et al., 2008). The lack of oxidative stress in these organ may be due to presence of metallothionein. Metallothionein is a protein that sequester heavy metals and render them non-toxic (Klaossen, 1978). It is a powerful interceptor of free radicals.

The lack of significant change in the level of Brain SOD of rats administered these metals and their combination may also be due to the Blood-brain barrier. The blood-brain barrier has been reported to restrict the entry of toxicant into the brain (Thimbrell, 1991).

Conversely, the increased level of kidney SOD in the metal exposed rats is an indication of oxidative stress occasioned by metal induced increased in free radicals. SOD is inducible and the level of this molecule increases as the need to protect cells from free radicals

increases (Lavelle et al., 1973). Similarly, increase in SOD in liver and kidney has been reported in male and female rats exposed to lead in drinking water (Algnazal et al., 2008).

Parallel analysis of catalase activity in plasma and tissue of rats treated with the metals (Table 4.3) indicate a decreased in level of catalase in the liver and testis but not in the other tissue. SOD and catalase work in tandem because they are functionally related since the product of SOD hydrogen peroxide is the substrate for catalase (Radjendirane et al., 1997) Thus the observed decrease in the level of catalase may reduce the ability of these tissues to handle metal induce increase in free radicals. However, it is noteworthy that catalase does not represent the major route of hydrogen peroxide detoxification (Doroshow et al., 1980).

The increased level of malondialdehyde in the testis of rats treated with cadmium plus arsenic (Table 4.4) is an indication of increased level of membrane lipid peroxidation occasioned by metal induced oxidative stress. This is corroborated by the corresponding decrease in catalase activity observed in this organ (Table 4.3). Available experimental data indicate that cells increase the production of antioxidant enzymes such as SOD and catalase in order to overcome oxidative stress (Gupta et al., 1991).

The alteration in the activities of AST and ALT (Tables 4.5 & 4.6) in the plasma, kidney, liver and heart of the metal exposed rats may result in alteration of amino acid metabolism. Amino transferases are involved in the catabolism of amino acid and the level of these enzymes may be altered under metal stress (Vinodhini and Narayana 2008). The metal induced damage to organs may give rise to increased level of amino transferases in the blood (El-Naga *et al*., 2005). Metals induced damage has been linked to membrane lipid peroxidation occasioned by oxidative stress (Gotz et al., 1994). However, examination of the data indicates no significant change in the level of malondialdehyde, a marker of lipid peroxidation (Table 4) in some of the organs of the metal exposed rats. Thus, this is evidence that there is no damage to the liver, kidney and heart despite the alteration of the amino transferases in the tissues. This may be attributed to the inability of these metals to be easily released from the metal incorporated fish in diet; hence it is less available invivo. This interpretation agrees with the view of Lind *et al* (1995). These authors showed that bioavailability of Cadmium from boiled crab hepatopancreas was slightly lower than that of mushroom and inorganic cadmium with fed to mice. The study revealed that cadmium in crab hepatopancreas is mainly associated with denatured proteins with low solubility.

Finally, examination of the data obtained indicates that both metals have comparable effect on some of the biochemical parameters in terms of their percentage changes. For example, this is obvious in the effect of both arsenic and cadmium on kidney SOD (Table 4.2); liver and testis catalase (Table 4.3); Testis malondialdehyde (Table 4.4); liver and heart AST (Table 4.5) and kidney ALT (Table 4.6). However, both metals appear to have antagonic interaction when consumed together. This is because their combined effect was less than each of their individual effects in kidney SOD (Table 4.2); liver catalase (Table 4.3); brain malondialdehyde (Table 4.4); liver and heart AST (Table 4.5) and kidney ALT (Table 4.6). These observations are in line with the observations of Vellinger et al (2012) who investigated antagonistic toxicity of arsenate and cadmium in fresh water amphipod (*Gammarua pulex*). Their result indicated that the antagonistic effect of the metals were lowest at a high concentration of both metals combined compared to individual metals. This claim were evidenced by the observed metal concentrations in muscle and other tissues of the fishes exposed t the metals in the study.

5.2.0 Conclusion

The present study showed that consumption of arsenic and cadmium through the food-chain for three month altered the activities of SOD, catalase and the amino transfereases. Conversely beside the testis, no significant change was observed in the level of malondialdehyde in the other organs of exposed rats.

5.3.0 Recommendation

From the work done, it is recommended that more works on food-chain mediated toxicity of arsenic, cadmium and other heavy metals should be carried out.

5.4.0 Contribution to Knowledge

1. The study revealed that consumption of arsenic and cadmium via the food-chain did not cause damage to most of the organs of rats which may be due to decreased bioavailability of these metals through the food-chain.

2. The study also revealed that arsenic and cadmium appear to be antagonistic when consumed together via the food chain and this may be responsible for their lower effects on some biochemical parameters as compared to when consumed alone.

References

Abernethy, D.R., DeStefano, A.J., Cecil, T.L., Zaidi, K. and Williams, R.L. (2010). Metal impurities in food and drugs.*Pharmaceut. Res.*, **27**(5): 750-755

Abou El Nga, E.H., El-Moselhy, K.M., Hamed, M.A (2005). Toxicity of Cadmium and Copper and their effects on some biochemical parameters of marine fish *Mugil seheli*. *Egyptian J. Aquat. Res*. **31**(2):60-71

Adam-Vizi, V. and Seregi, M. (1982). Receptor dependent stimulatory effect of noradrenaline on Na^+/K^+ ATPase in rat brain homogenate: Role of Lipid peroxidation. *Biochem.Pharmacol*., 31 :2231-2236.

Aebi, H. (1984). Aebi, H. ed. "Catalase in vitro". *Meth. Enzymol.*. Methods in Enzymology **105**: 121–126.

Agency for Toxic Substances and Disease Registry (1989). *Toxicological profile for cadmium*. ATSDR/U.S. Public Health Service, Atlanta/TP-88/08.

Agency for Toxic Substances and Disease Registry (1999). *Toxicological profile for Cadmium*. ATSDR / US department of Health and Human Services, Atlanta/, US.

Agency for Toxic Substances and Disease Registry (ATSDR) (1991). Toxicological profile for cadmium, draft for public comment. Public Health Service, U.S. Department of Health and Human Services, October 1991.

Agency for Toxic Substances and Disease Registry (ATSDR) (1997). Toxicological profile for cadmium, draft for public comment (update). Public Health Service, U.S. Department of Health and Human Services, June 2011.

Agency for Toxic Substances and Disease Registry (ATSDR) (1997). Toxicological profile for cadmium, draft for public comment (update). Public Health Service, U.S. Department of Health and Human Services, September 1997.

Ahmad, S., Kitchin, K.T., Cullen, W.R., 2000. Arsenic species that cause release of iron from ferritin and generation of activated oxygen. Arch. Biochem. Biophys. 382, 195–202.

Ahokas, R., Dilts, P. (1979) Cadmium uptake by the rat embryo as a function of gestational age. *Am. J. Obstet. Gynecol*., **135**, 219–222.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K, and Walter, P. (2002). "Peroxisomes. *Molecular Biology of the Cell* (4th ed.). New York: Garland Science.

Al-Fartosi, K.G., Al-Taae, H.I., Al-Haider, M.N., Hussein, K.A., Sachit, F., Rashid, L. (2010). Cadmium acetate induced hematotoxicity, sperm abnormality and mutagenicity in rats. *J.Thi-Qar Sci.*, **2** (1).

Alghazal M., Sultiakova, I., Kovalkovicoca, N., Legath, J., Falis, M. and Pish J. (2008). Induction of Macronuclei in Rats Bone after Chronic Exposure to Lead Acetate Trihydrate. *Toxicol. Ind. Health,* 24:587-593

Amo, T., Atomi, H. and Imanaka, T. (2002). "Unique presence of a manganese catalase in a hyperthermophilic archaeon, Pyrobaculum calidifontis VA1". *J. Bacteriol.* **184** (12): 3305–3312.

Anane, R. and Creppy E.E. (2001). Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase and catalase and vitamins E and C. Hum. *Exp. Toxicol.*, **20**: 477-481.

Andersen, O., Nielsen, J.B. and Svendsen, P. (1988). Oral cadmium chloride intoxication in mice: Effects of dose on tissue damage, intestinal absorption and relative organ distribution. *Toxicology* **48**:225-236.

Angstadt, and Carol N. (1997). Purine and Pyrimidine Metabolism: Purine Catabolism. NetBiochem.

Annino, J.S. and Giese, R.W (1976). *Clinical chemistry*, *principles and procedures,* 4th edition*,* Little Brown and Company, Boston. pp. 76-82

Antman, Karen H. (2001). "The History of Arsenic Trioxide in Cancer Therapy". *The oncologist. 6 (Suppl 2): 1–2.*

Asagba, S.O and Eriyamremu, (2007). Oral cadmium exposure alters haemological and liver function parameters of rats fed a Nigerian-Like Diet. *J. Nultr. Environ. Med.***16**(3-4):267-274

Asagba, S.O. (2010). Alteration in the activity of oxidative enzymes in the tissues of male wistar albino rats exposed to cadmium. *IJOMEH***,** 23(1):55 – 62.

Asagba, S.O., Eriyamremu, G.E., Adaikpoh, M.A. and Ezeoma, A. (2005). Levels of lipid peroxidation, superoxide dismutase and Na+/K+-ATPasa in some tissues of rats exposed to a Nigerian-like diet and cadmium. *Biol. Trace Elem. Res*. **100** (1): 143-154.

Ashraf, M.W (2012). Levels of Heavy Metals in Popular Cigarette Brands and Exposure to These Metals via Smoking *The Scientific World Journal,* 2012: 729430

Ayres, R. U., Ayres, L. and Råde, I. (2003) *The Life Cycle of Copper, Its Co-Products and Byproducts* . Springer. pp. 135–141.

Bagchi, O., Bagchi, M., Hassoun, E.A. and Stohs, S.J (1996). Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague- Dawley rats. *Biol. Trace. Elem. Res.* 52:143-154

Baillie, J.K., Bates, M.G., Thompson, A.A., Waring, W.S., Partridge, R.W., Schnopp, M.F., Simpson, A., Gulliver-Sloan, F., Maxwell, S.R. and Webb D.J. (2007). Endogenous urate production augments plasma antioxidant capacity in healthy lowland subjects exposed to high altitude. *Chest*, **131** (5): 1473–1478.

Bartoszek, M. & Sulkowski, W.W (2006).The Study of pH Influence on Bovine Liver Catalase by Means of UV-VIS Spectroscopy and Spin Labelling Method. *Polish J. of Environ. Stud.* **15**(4A):41-43

Benoff, S., Auborn, K., Marmar, J.L. and Hurley, I.R. (2008) Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Elsevier Inc.* **89**(1): 73-79.

Berg, J.M., Tymoczko, J.L. and Stryer, L (2006). Biochemistry. W.H. Freeman. pp. 656–660.

Bernard, A. and Lauwerys, R. (1986). Effects of cadmium exposure in humans. In: *Handbook of experimental pharmacology*, Foulkes, E.C. eds Berlin: Springer-Verlag,. p. 135-77.

Bernard, A.M. and Lauwerys, R. (1984) Cadmium in human population. Experimentia **40**:143-152.

Beton, D.C., Andrews, G.S. and Davies, H.J. (1966). Acute cadmium fume poisoning; five cases with one death from renal necrosis. *Br. J. Ind. Med.* **23**:292.

Beyer, W., Imay, J., and Fridovich, I. (1991). Superoxide dismutases. *Prog. Nucl. Acid Res.* 40:221-253.

Beyer, W., Imlay, J. and Fridovich, I. (1991). SODs varieties and distributions: x-ray crystallography of Mn-SODs and Fe-SODs. *Prog. Nucleic Acid Res. Mol. Biol.*, **40**: 221-253.

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. Jama-J Am Med Assoc 297(8):842–857

Boon, E.M., Downs, A. and Marcey, D. (2007). "Proposed Mechanism of Catalase" (http:/ / biology. kenyon. edu/ BMB/ Chime/ catalase/ frames/ cattx. htm#Proposed Mechanism of Catalase). *Catalase: H_2O_2: H_2O_2 Oxidoreductase: Catalase Structural Tutorial*.

Burdun, G. D. (1958). On the new determination of the meter" . *Measurement Techniques* **1**(3): 259–264.

Candelaria, M., Garcia-Arias, A., Cetina, L. and Dueñas-Gonzalez, A. (2006). Radio sensitizers in cervical cancer: Cisplatin and beyond. *Radiat Oncol*, **1**: 15-32.

Cardenas, A., Bernard, A., Lauwerys, R. (1992). Incorporation of [35S] sulfate into glomerular membranes of rats chronically exposed to cadmium and its relation with urinary glycosaminoglycans and proteinuria. *Toxicol.*, **76**:219-231.

Casalino, E., Calzaretti, G., Sblano, C. and Landriscina, C. (2002) Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicol.* **179**:37–50.

Casalino, E., Sblano, C. and Landriscina, C. (1997) Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. *Arch. Biochem. Biophys*. **346**: 171-179.

Chaube, S., Nishimura, H. and Swinyard, C. (1973). Zinc and cadmium in normal human embryos and fetuses. *Arch. Environ. Health*, **26**, 237–240.

Chelikani, P., Fita, I. and Loewen, P.C. (2004). "Diversity of structures and properties among catalases". *Cell. Mol. Life Sci.* **61** (2):192–208.

Clark, D. and Sokoloff, L. (1999), *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, Lippincott, pp. 637–670.

Clarkson, T. (1995). Health effects of metals: a role for evolution. *Environ. Health Pespect.* **105** (1): 9-12.

De Burbure, C., Buchet, J.P., Leroyer, A., Nisse, C., Haguenoer, J.M. and Mutti, A. (2006). Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. *Environ. Health Perspect.* **114**, 584–590.

Dhalla, N.S., Temah, R.M. and Netticadan, T. (2001). Role of oxidative stress in cardiovascular diseases. *J. Hypertens*., **18**: 655.

Doroshow, J. H., Locker, G.Y and Myer, C.E (1980). Enzymatic Defense of the Mouse Heart Against Reactive Oxygen Metabolites: Alteration Produced by Doxorubicin. *J. Clin. Invest.* 65:128-135

Dudley, R.E., Gammal, L.M. and Klaassen, C.D. (1985). Cadmium-induced hepatic and renal injury in chronically exposed rats: Likely role of hepatic cadmium-metallothionein in nephrotoxicity. *Toxicol. Appl. Pharmacol.*, **77**:414-426.

Eisler,R. (2000). *Cadmium. In: Handbook of chemical risk assessment.* (Government, U.S.Ed) CRC press, Florida, 1-43.

El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S. and Baghdadi, H.H. (2004). Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and B-carotene. Food Chem. Toxicol. **42**: 1563–71.

Ellington, W. R. and Long, G. L. (1978) Purification and characterization of a highly unusual tetrameric o-lactate dehydrogenase from the muscle of the giant barnacle Balanus nubilus Darwin. *Archiv. Biochem. Biophys.* **186.** 265-274

Elsenhans, B., Strugala, G., Schmann, K. (1999). Longitudinal pattern of enzymatic and adsorptive functions in small intestine of rats after short term exposure to dietary cadmium chloride. *Arch. Environ. Contam. Toxicol.* 36: 341-346.

Eriyamremu, G.E., Asagba, S.O., Onyeke, E.C. and Adaikpo, M.A (2005). Changes in carboxypeptidase A, dipeptidase and NA+/K+ ATPase activities in the intestine of rats orally exposed to different doses of cadmium. *Biometals* 18:1-6

European Commision (EU) (2009). *Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food.* The EFSA Journal *(*2009*) 980, 1–139.*

Faro, Andre Ricardo Maia da Costa de, Pinto, Wagner de Jesus, Ferreira, Aldo Pacheco, Barbosa Junior, Fernando, Souza, Vanessa Cristina de Oliveira, Fujimoto, Denys Eiti, Koifman, Rosalina Jorge, & Koifman, Sergio. (2014). Serum cadmium levels in a sample of blood donors in the Western Amazon, Brazil, 2010-2011. *Cadernos de Saúde Pública*, *30*(2), 403-414.

Fleischer, M., Cabri, L. J., Chao, G. Y. and Pabst, A. (1980) "New Mineral Names" . *American Mineralogist* **65**: 1065–1070.

Flora, S.J.S., Megha, M. and Ashish, M. (2008). Heavy metal induced oxidative stress and its possible reversal by chelation Therapy. *Indian. J. Med. Res.* 128:501-523

Foulkes, E.C. (1978). Renal tubular transport of cadmium-metallothionein. *Toxicol. Appl. Pharmacol.*, 45:505-512.

Foulkes, E.C. (1984). Intestinal absorption of heavy metals. In: Csaky TZ, ed. *Handbook of experimental pharmacology*. Vol. 70/I. Pharmacology of intestinal permeation. Berlin: Springer Verlag, 543-565.

Foulkes, E.C. (1985). Interactions between metals in rat jejunum: Implications on the nature of cadmium uptake. *Toxicology* **37**:117-125.

Foulkes, E.C. (1990). The concept of critical levels of toxic heavy metals in target tissues. *CRC. Crit. Rev. Toxicol.*, **20**:327-339.

Fox, M.R.S. (1983). Cadmium bioavailability. *Federation Proc*. **42**, 1726-1729.

Friberg, L. (1950). Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning. *Acta. Med. Stand.* **138**(Suppl 240):1-124.

Friberg, L., Elinder, C., Kjellstrom, T. and Nordberg, G. (1985) *Cadmium and Health, A Toxicological and Epidemiological Appraisal*, Vol. I, *Exposure, Dose and Metabolism*, Cleveland, OH, CRC Press.

Friberg, L., Elinder, C.G., Kjellstrom, T. and Nordberg G. (1985) *Cadmium and Health: A Toxicological and Epidemiological Appraisal,* CRC Press, Baca Raton, Florida. 23 – 63.

Friberg, L., Nordberg, G.F. and Vouk V.B., eds. (1986) *Handbook of the toxicology of metals.* Vol. II. Amsterdam, Elsevier, pp. 130–184.

Fthenakis, V. M. (2004). "Life cycle impact analysis of cadmium in CdTe PV production". *Renewable and Sustainable Energy Reviews* **8** (4): 303.

Gaetani, G., Ferraris, A., Rolfo, M., Mangerini, R., Arena, S. and Kirkman, H. (1996). "Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes". *Blood* **87** (4): 1595–9.

Gaurav, D., Preet, S. and Dua, K. K. (2010) Chronic cadmium toxicity in rats: treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. *Journal of Food and Drug Analysis*, **18**(6): 464-470.

Gaurav, D., Preet, S. and Dua, K.K. (2010). Chronic cadmium toxicity in rats: Treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. *J. Food Drug Anal.*, **18**: 464-470.

Gaze, D.C. (2007). "The role of existing and novel cardiac biomarkers for cardioprotection". *Curr. Opin. Invest. Drugs*, **8** (9): 711–7.

Ghouri, N., Preiss, D. and Sattar, N. (2010). Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology,* 52:1156-1161.

Giannini, E., Botta, F., Fasoli, A., Ceppa, P., Risso, D., Lantieri, P.B., Celle, G. and Testa R. (1999). Progressive liver functional impairment is associated with an increase in AST/ALT ratio. *Dig Dis Sci*., 44:1249-1253.

Gibaud, Stéphane; Jaouen, Gérard (2010). "Arsenic – based drugs: from Fowler's solution to modern anticancer chemotherapy". Topics in Organometallic Chemistry. Topics in Organometallic Chemistry. 32: 1–20.

Gill, K.D., Pal, R., Sandhir, R., et al. (1989). Effect of chronic cadmium exposure on lipid composition and peroxidation in liver and kidneys in rats. *Med. Sci. Res.* **17**:921-924.

Globus, J.H., Ginsburg, S.W., 1933. Pericapillary encephalorrhagia due to arsphenamine. Arch. Neurol. Psychiatry 30, 1226-1247.

Goessling, W., Massaro, J.M., Vasan, R.S., D'Agostino, R.B., Ellison, R.C. and Fox, C.S. (2008). Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology*, **135**: 1935-1944, 1944.

Gonick, H.C. (2008). Nephrotoxicity of cadmium & lead. *Indian J. Med. Res.*, **128**: 335-352.

González-Cortijo, L., Carballo, N. and González-Martín A. (2008). Novel chemotherapy approaches in chemoradiation protocols. *Gynecol Oncol,* **110**: 45-8.

Goon, D., Klaassen, C.D. (1989). Dosage-dependent absorption of cadmium in the rat intestine measured *in situ. Toxicol. Appl. Pharmacol.*, **100**:41-50.

Gotz, M.E., Kunig, G., Riederer, G.P., Youdin, M.B.H (1994). Oxidative Stress: Free Radicals Production in Neural Degeneration. *Pharmaceut. Therapeut,* 63:37-122.

Goyer, R.A., Cherian, M.G. and Delaquerriere-Richardson, L. (1984). Correlation of parameters of cadmium exposure with onset of cadmium-induced nephropathy in rats. *J. Environ. Pathol. Toxicol. Oncol.*, **5**:89-100.

Goyer, R.A., Epstein, S., Bhattacharyya, M. and Pounds, J. (1994) Environmental risk factors for osteoporosis. *Environ. Health Perspectives*, **102**, 390–394.

Grosicki, A. and Kowalski, B. (2002). Whole body and organ retention of cadmium after repeated administration to rats. *Bull. Vet. Inst. Pullawy.,* 46:143-147

Groten, J.P., Sinkeldam, E.l. and Luten, J.B. (1990). Comparison of the toxicity of inorganic and liver-incorporated cadmium: A 4-week feeding study in rats. *Food Chem. Toxicol.*, **28**:435-441.

Guéraud, F., Atalay, M., Bresgen, N., Cipak, A., Eckl, P.M., Huc, L., Jouanin, I., Siems, W. and Uchida, K. (2010). Chemistry and biochemistry of lipid peroxidation products: *Free Rad. Res*., **44**(10):1098-1124.

Guilherminol, L., Soares A.M.V.M., Carvalho, A.P. and Lopes, M.C (1998). Effect of cadmium and parathion exposure on heamatology and blood biochemistry of adult male rats. *Bull. Environ. Contam. Toxicol.* 60:52-59

Gupta, S., Arhar, M., Behari, J.R and Srivastava, R.C (1991). Cadmium mediated induction of cellular defense mechanism: A novel example for development of adaptive response against a toxicant. *Ind. Health.* 29:1-9

Gutteridge, J.M.C. and Wilkins, C. (1982). Copper dependent hydroxyl radical damage to ascorbic acid. Formation of a thiobarbiturie acid reactive products. *FEBS Lett*. 137:327-340.

Hallenbeck, W (1984). Human health effects of exposure to cadmium. *Experientia* **40**, 136-142.

Hayashi, H., Wada, H., Yoshimura, T., Esaki, N. and Soda K (1990). "Recent topics in pyridoxal 5'-phosphate enzyme studies". *Annu. Rev. Biochem.*, **59**: 87–110.

Henry, J.B. (1979). Clinical diagnosis and management by laboratory methods. W.B. Saunders and Company, Philadelphia, PA, p. 365.

Hensel, R., Mayr, U., Lins, C. and Kandler, 0. (1981) Amino acid sequence of a dodecapeptide from the substrate binding region of the L-lactate dehydrogenase from Lactobacillus curvarus, Lactobacil- */us xylosus***,** and Bacillus stereothermophilus. Hoppe-Seyler's *Z. Physiol. Chem*. **362**: 1031-1036

Hida, T., Kozaki, K. and Ito, H. (2002). Significant growth inhibition of human lung cancer cells both in vitro and in vivo by the combined use of a selective cyclooxygenase 2 inhibitor, JTE-522, and conventional anticancer agents. *Clin Cancer Res*, **8**: 2443-7.

Hida, T., Kozaki, K. and Muramatsu, H. (2000). Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res*, **6**: 2006-11.

Hijova, E., Nistiar, F. and Kuchta M. (2004) Influence of acute cadmium exposure on plasma antioxidant parameters in rats. *Bull Vet Inst Pulawy.* **48**, 155-157.

Hille, R. (2005). Molybdenum-containing hydroxylases. *Archives of biochemistry and biophysics* **433** (1): 107–16.

Hirotsu, K., Goto, M., Okamoto, A. and Miyahara, I. (2005). Dual substrate recognition of aminotransferases. *Chem. Rec.*, **5**(3): 160-172.

Ho, Y.S., Xiong, Y., Ma, W., Spector, A. and Ho, D. (2004). "Mice Lacking Catalase Develop Normally but Show Differential Sensitivity to Oxidant Tissue Injury". *J Biol Chem* **279** (31): 32804–32812.

Hodgman, C.D., Weast, R.C., Shankland, R.S. and Selby S.M. (1961). *Handbook of Chemistry and Physics, 43rd Edition.* Chemical Rubber Publishing Company, Cleveland.

Holleman, A. F., Wiberg E., Wiberg, Nils, (1985). "Cadmium". *Lehrbuch der Anorganischen Chemie, 91–100*. Walter de Gruyter. pp. 1056–1057.

Holleman, Arnold F.; Wiberg, Egon; Wiberg, Nils (1985). "Chromium". Lehrbuch der Anorganischen Chemie (in German) (91–100 ed.). Walter de Gruyter. pp. 1081–1095. ISBN 3-11-007511-3.

Hori, N., Uehara, K. and Mikami, Y. (1992). Enzymatic Synthesis of 5-Methyluridine from Adenosine and Thymine with High Efficiency". *Biosci. Biotechnol. Biochem.*, **56** (4): 580–582.

Huet, P. M.; Guillaume, E.; Cote, J.; Légaré, A.; Lavoie, P.; Viallet, A. (1975). "Noncirrhotic presinusoidal portal hypertension associated with chronic arsenical intoxication". *Gastroenterology*. 68 (5 Pt 1): 1270–1277.

IARC (1976) *Cadmium, nickel, some epoxides, miscellaneous industrial chemicals and general considerations on volatile anaesthetics*. Lyon, International Agency for Research on Cancer, pp. 39–74 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 11).

IARC (1987) *Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1–42*. Lyon, International Agency for Research on Cancer, pp. 139–142 (IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Suppl. 7).

Iglesias, M.T., De Lorenzo, C., Polo, M., Martín-Alvares, P.J. and Pueyo E (2004). Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *J. Agric. Food Chem*., **52**: 84-89.

Ikeda, M., Zang, Z.W., Moon, C.S., Shimbo, S., Watanabe, T., Nakatsuka, H., MatsudaInoguchi, N. and Higashikawa, K. (2000). Possible effects of environmental cadmium exposure on kidney function in the Japanese general population. *Int. Arch. Occup. Environ. Health*. **73**: 15 - 25**.**

Iscan, M., Coban, T. and Eke, B.C. (1994). Differential combined effect of cadmium and nickel on hepatic and renal glutathione S-transferase of the guinea pig. *Environ Health Perspect,* **102**: 69-72.

Jakubowski, M., Trojanowska, B., Kowalska, G., Gendek, E., Starzyfiski, Z., Krajewska, B. and Jajte, J. (1987). Occupational exposure to cadmium and kidney dysfunction. *Int. Arch. Occup. Environ. Health*, *59:567-577*

Jarup, K., Berglund, M., Elinder, C., Nordberg, G. and Vahter, M. (1998b) Health effects of cadmium exposure—A review of the literature and a risk estimate. *Scand. J. Work Environ. Health.*, **24** (Suppl. 1).

Jarup, L., Alfven, T., Persson, B., Toss, G. and Elinder, C.G. (1998a) Cadmium may be a risk factor for osteoporosis. *Occup. Environ. Med*., **55**, 435–439.

JECFA (1989). *Toxicological evaluation of certain food additives and contaminants*. Geneva, World Health Organization, Joint FAO/WHO Expert Committee on Food Additives; Cambridge, Cambridge University Press, pp. 163–219.

JECFA (1993) *Evaluation of certain food additives and contaminants: forty-first report of the Joint FAO/WHO Expert Committee on Food Additives.* Geneva, World Health Organization, Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series No. 837).

JECFA (2000a) *Cadmium*. Geneva, World Health Organization, Joint FAO/WHO Expert Committee on Food Additives (WHO Food Additives Series No. 46).

JECFA (2000b) *Summary and conclusions of the fifty-fifth meeting, Geneva, 6–15 June 2000*. Geneva, World Health Organization, Joint FAO/WHO Expert Committee on Food Additives.

JECFA (2011) *Evaluation of certain food additives and contaminants: seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives.* Geneva, World Health Organization, Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series No. 960).

Jedrzejczak, P., Taszarek-Hauke, G., Derwich, K., Depa, M. and Pawelczyk, L. (2004). The sperm quality in fertile smokers. *Przegl. Lek.*, **61**: 1028–30.

Jennewein, Marc; Lewis, M. A.; Zhao, D.; Tsyganov, E.; Slavine, N.; He, J.; Watkins, L.; Kodibagkar, V. D.; O'Kelly, S.; Kulkarni, P.; Antich, P.; Hermanne, A.; Rösch, F.; Mason, R.; Thorpe, Ph. (2008). *"Vascular Imaging of Solid Tumors in Rats with a Radioactive Arsenic-Labeled Antibody that Binds Exposed Phosphatidylserine"*. Journal of Clinical Cancer. 14 (5): 1377–1385

Jiang, H.M., Guo, H. and Zhu, H. (1990) Clinical significance of hair cadmium content in the diagnosis of mental retardation of children. *Chin. Med. J.*, **103**, 331–334.

Jolliffe, D.M., Budd, J. and Gwilt, J., (1991). Massive acute arsenic poisoning. Anesthesia **46**: 288–290.

Jones, F. T. (2007). "A Broad View of Arsenic". Poultry Science*. 86 (1): 2–14.*

Jung, K., Pergrande, M., Graubaum, H.C., Fels, L.M., Endl, U. and Stolte, H. (1993) Urinary proteins and enzymes as early indicators of renal dysfunction in chronic exposure to cadmium. *Clin. Chem*., **39**, 757–765.

Kamel, M.M., Abd El Razek, A.H., Ahmed, K.A. and Kamel, G.M. (2011). Exposure of Adult Male Rats to Cadmium: Assessment of Sexual Behaviour, Fertility, Aggression as well as Anxiety like Behaviour with Special Reference to Biochemical and Pathological Alterations. *Life Science Journal*, **8**(2): 106-119.

Kirsch, J.F., Eichele, G., Ford, G., Vincent, M.G., Jansonius, J.N., Gehring, H. et al. (1984). "Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure". *J. Mol. Biol.*, **174** (3): 497–525.

Kjellstrom, T. and Nordberg, G.F. (1978). A kinetic model of cadmium metabolism in the human being. *Environ. Res.*, **16**:248-269.

Kjellstrom, T. and Nordberg, G.F. (1985). Kinetic model of cadmium metabolism. In: Friberg L, Elinder CG, Kjellstrom T, et al., eds. *Cadmium and health: A toxicological and epidemiological appraisal.* Vol. I. Exposure, dose and metabolism. Boca Raton, FL: CRC Press, 179-197.

Kjellstrom, T., Borg, K. and Lind, B. (1978). Cadmium in feces as an estimator of daily cadmium intake in Sweden. *Environ. Res.* **15**:242-251.

Klaassen, C.D., (1978). Effect of Mettallothionein on Hepatic Disposition of Metals. *Am. J. Physiol*., 234: E47-E53

Klos, A. (2001). Lead, cadmium and mercury content in meals planned for consumption in selected kindergartens in Warsaw. IV International Scientific - Technical Conference Warsaw 4-5 X.

Kochhar, S. and Christen, P. (1992). "Mechanism of racemization of amino acids by aspartate aminotransferase". *Eur J Biochem* **203** (3): 563–569.

Koizumi, T., Shirakura, G., Kumagai, H., Tatsumoto, H. and Suzuki, K.T. (1996). Mechanism of cadmium-induced cytotoxicity in rat hepatocytes: cadmium-induced active oxygen-related permeability changes of the plasma membrane. *Toxicology*, **114**: 124-134.

Kotsonis, F.N. and Klaassen, C.D. (1978). The relationship of metallothionein to the toxicity of cadmium after prolonged administration to rats. *Toxicol. Appl. Phamacol.*, **46**:39-54.

Kowalczyk, E., Anna, K., Pawel, F., Maria, K., Jan, N., Jan, B., Jozef, K. and Piotr, T. (2003). Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. *Acta. Biochemical. Polonica.* **50**(2): 543-548.

Kowalczyk, E., Jankowski, A., Niedworok, J., OEmigielski, J. and Tyoelerowicz, P. (2002) Effect of long-term cadmium intoxication on selected biochemical parameters in experimental animals. *Pol. J. Environ. Stud*.; **11**: 599–601.

Koyu, A., Gokcimen, A., Ozguner, F., Bayram, D.S. and Kocak A. (2006) Evaluation of the effects of cadmium on rat liver. *Mol. Cell. Biochem*. **284**: 81-5.

Krajnc, E.I. (1987) *Integrated criteria document. Cadmium — Effects. Appendix*. Bilthoven, National Institute of Public Health and Environmental Protection (Report No. 758476004).

Kreis, I.A., Wijga, A. and van Wijnen, J.H. (1992). Assessment of the lifetime accumulated cadmium intake from food in Kempenland. *Sci. Total Environ*., **127**, 281–292.

Krichah, R., Rhouma, K.B., Hallègue, D., Tébourbi, O., Joulin, V., Couton, D. and Sakly M. (2003). Acute Cadmium Administration Induces Apoptosis in Rat Thymus and Testicle, but not Liver. *Polish J. Environ. Studies,* **12**(5): 589-594.

Kuester, R.K., Waalkes, M.P., Goering, P.L., Fishers, B.L., Micuskey, R.S., Sipes, I.G (2002). Differential hepatotoxicity induced by cadmium in fisher 344 and Sprague-dawley rats. *Toxicol*. *Sci*. 65:51-159

Kumagai, Yoshito; Sumi, Daigo (2007). "Arsenic: Signal Transduction, Transcription Factor, and Biotransformation Involved in Cellular Response and Toxicity". Annual Review of Pharmacology and Toxicology. 47: 243-262.

Lafuente, A., Gonzalez-Carracedo, A., Romero, A., and Esquifino, A.I. (2003). Effect of cadmium on 24-h variations in hypothalamic dopamine and serotonin metabolism in adult male rats. *Exp. Brain Res.* 149, 200–206.

Lansche, A. M. (1956). "Minerals Yearbook, Volume I: Metals and Minerals (Except Fuels)" . United States Geological Survey. Retrieved April 21, 2008.

László, G. (2008). Catalase Deficiency and Type 2 Diabetes". *Diabetes Care* **24** (10): e93–e93.

László, G., Ágota, L. and William, N. B. (2001). "Blood Catalase Deficiency and Diabetes in Hungary". *Diabetes Care* **24** (10): 1839–1840.

Lavelle, F., Michelson, A.M. and Dimitrijevic, L. (1973). Biological protection by superoxide dismutase. *Biochem*. *Biophys*. *Res*. *Commun*. **55**(2):350-357

Lee, J.S. andWhite, K.L. (1980). A review of health effects of cadmium. *Am. J. Ind. Med.,* **1**: 307-317.

Lehman, L.D. and Klaassen, C.D. (1986). Dosage-dependent disposition of cadmium administered orally to rats. *Toxicol. Appl. Pharmacol.*, **84**:159-167.

Leret, M.L., Millan, J.A., and Antonio, M.T. (2003). Perinatal exposure to lead and cadmium affects anxiety- like behaviour. *Toxicology* 186, 125– 130.

Lide, D. R., (2005). Magnetic susceptibility of the elements and inorganic compounds. (86th ed.). Boca Raton (FL): CRC Press.

Lind, Y.A., Wicklund, G., Engman, J. and Johem, J. (1995). Bioavalaibility of Cadmium from Crab Hepatopancreas and mushroom in relation to inorganic cadmium: a 9 week feeding study in mice. *Food. Chem. Toxicol*. 33:667-673

Loew, O. (1900). "A New Enzyme of General Occurrence in Organisms". *Science* **11** (279): 701–702.

Lopez, E., Arce, C., Oset-Gasque, M. J., Canadas, S., and Gonzalez, M.P. (2006). Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. *Free Radic. Biol. Med.* **40,** 940–951.

Lucas, P.A., Jariwalla, A.G., Jones, J.H., (1980). Fatal cadmium fumes inhalation. *Lancet* :205.

Maass, E. (1998). "How does the concentration of hydrogen peroxide affect the reaction". MadSci Network.

Madaan, N. and Mudgal, V. (2009). Differential tolerance behaviour of safflower accessions to some heavy metals. *Int J Appl Environ Sci*, **4**(4): 413-20.

Maehly, A. and Chance, B. (1954). The assay of catalases and peroxidases". *Methods Biochem. Anal*., Methods of Biochemical Analysis **1**:357–424.

Manca, D., Ricard, A.C., Trottier, B. and Chevalier, G. (1991). Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. *Toxicology,* **67**: 303-23.

Martelli, A., Rousselet, E., Dycke,C., Bouron, A. and moulis, J.M. (2006). Cadmium toxicity in animal cells by interference with essential metals.*Biochimie*. 88:1807-1814.

Masaoka, T., Akaori, F. and Arai, S. (1994). A nine year chronic toxicity study of cadmium ingestion in monkeys. Effects of dietary cadmium on the general health of monkeys. *Vet. Hum. Toxicol*., **36**, 189–194.

Mazzio, E.A. and Soliman, K.F.A. (2009). In vitro screening for the tumoricidal properties of International medicinal herbs. *Phytother Res*, **23**: 385-98.

Misra, H. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem.* **247:** 3170-3175.

Mladenovic, D., Tatjana, R., Milica, N., Danijela, V., Tamara, C., Grbatinicci and Rada, J.V. (2010). Possible Role Of Oxidative Stress In Acute Cadmium Hepatotoxicity In Rats. *Acta. Veterinaria. (Beograd)*, **60**(5-6): 449-459.

Morrow, H. (2010). "Cadmium and Cadmium Alloys". *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons. pp. 1–36.

Moss, D.W. and Henderson, A.R. (1986). Enzymes. *In*: Burtis, C.A., Ashwood, E.R., eds. Tietz Textbook of Clinical Chemistry. 2nd edn. Philadelphia, Saunders Co., pp. 735–896.

Mudgal, V., Madaan, N. and Mudgal, A. (2010). Heavy metals in plants: phytoremediation: plants used to remediate heavy metal pollution. *Agric Biol J*, **1**(1): 40-6.

Murthy, M.R., Reid, T.J., Sicignano, A., Tanaka, N. and Rossmann, M.G. (1981). "Structure of beef liver catalase". *J. Mol. Biol.* **152** (2): 465–499.

Nachman, Keeve E.; Graham, Jay P.; Price, Lance B.; Silbergeld, Ellen K. (2005). "Arsenic: A Roadblock to Potential Animal Waste Management Solutions". Environmental Health Perspectives. 113 (9): 1123–1124.

Nordberg, G., Nogawa, K., Nordberg, M. and Friberg, L. (2007) Cadmium. In: *Handbook on toxicology of metals*. Nordberg, G., Fowler, B., Nordberg, M. and Friberg, L. (eds) New York: Academic Press, p. 65-78.

Nsiah, K., Dzogbefia, V.P., Ansong, D. Akoto, A.O., Boateng, H. and Ocloo, D. (2011). Pattern of AST and ALT Changes in Relation to Hemolysis in Sickle Cell Disease. *Clin. Med. Insights: Blood Disor.,* 4:1–9

Oba, K. and Uritani, I. (1982) L-Lactate dehydrogenase isozymes from sweet potato roots in Methods in Enzymology (Wood, W. A,, Ed.) Academic Press, New York and London, Vol. 89, pp 345-351

O'Brien, P. and Salasinski, H.J. (1998). Evidence that the reactions of cadmium in the presence of metallothionein can produce hydroxyl radicals. *Arch. Toxicol*., **72**: 690-700.

Oldereid, N.B., Thomassen, Y., Attramadal, A., Olaisen, B. and Purvis, K. (2003). Concentrations of lead, cadmium, and zinc in the tissues of reproductive organs of men. *J. Reprod. Fertil.,* **99**:421–5.

Oldiges, H., Hochrainer, D. and Glaser, U. (1989). Long-term inhalation study with Wistar rats and four cadmium compounds. *Toxi. Environ. Chem*. **19**, 217-222.

Osterberg, A.E., Kernohan, J.W., 1934. The presence of arsenic in the brain and its relation to pericapillary hemorrhages or so-called acute hemorrhagic encephalitis. Am. J. Clin. Pathol. 4, 362–369.

Patwardham, J.R. and Finckh, E.S. (1976). Fatal cadmium-fume pneumonitis. *Med. J. Aust.*, **1**:962-966.

Pershagen, G. (1983). The epidemiology of human arsenic exposure. In: Biological and environmental effects of arsenic, B.A Flower, (Ed), Elsevier, Amsterdam. 193–233.

Petering, H.G., Choudhury, H. and Stemmer, K.L. (1979). Some effects of oral ingestion of cadmium on zinc, copper and iron metabolism. *Environ. Health. Perspect.*, **28**:97-106.

Pratt, D.S and Kaplan, M.M (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med*. **342**(17):1266-1271.

Prescott, L., Harley, J.P. and Klein D.A. (1999). *Microbiology*. Boston: WCB/McGraw-Hill.

Radjendirane, V., Joseph, P., Jaishal, A.K. (1997). Gene expression of DT-diaphorase (NQ01) in cancer cells. In Forman, H.J and Cadenas. E. (Eds). *Oxidative Stress and Signal Transduction.* New York: Chapman and Hall; Pp. 441-475

Reels, H., Bernard, A.M., Cardenas, A., et al. (1993). Markers of early renal changes induced by industrial pollutants. III. Application to workers exposed to cadmium. *Brit. J. Ind. Med.* **50**:37-48.

Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and gllutamic pyruvic transaminases. *Am. J. Clin. Path.*, **28**: 56

Rikans, L.E. and Yamano, T. (2000). Mechanisms of cadmium-mediated acute hepatotoxicity. *J. biochem. mol. Toxicol*., **14**: 110-117.

Rodr´ıguez, V.M., Jiménez-Capdeville,M.E and Giordano, M.(2003). The effects of arsenic exposure on the nervous system Toxicology Letters 145 (2003) 1–18

Rodriguez, V.M., Jiménez-Capdeville, M.E. and Giordano, M. (2003). The effects of arsenic exposure on the nervous system. *Toxicology Letters,* **145**: 1–18.

Ros, J.P.M. and Slooff, W., eds. (1987) *Integrated criteria document. Cadmium*. Bilthoven, National Institute of Public Health and Environmental Protection (Report No. 758476004).

Roseman, E., Aring, C.D., 1941. Encephalopathy following neoarsphenamine therapy. N. Engl. J. Med. 24, 550–553.

Roy, P. and Saha, A. (2002). Metabolism and toxicity of arsenic: a human carcinogen. *Curr. Sci.,* **82** (1): 38-45.

Sabina C. Grund, Kunibert Hanusch, Hans Uwe Wolf (2005). "Arsenic and Arsenic Compounds", Ullmann's Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH, doi:10.1002/14356007.a03_113.pub2

Sakurai T (2003). "Biomethylation of Arsenic is Essentially Detoxicating Even*t"*. Journal of Health Science. 49 (3):171–178.

Saldivar, A. & Soto, V. (2009). Arsenic: An Abundant Natural Poison. *ProQuest Discovery Guides* http://www.csa.com/discoveryguides/discoveryguides-main.php

Sarkar, S., Yadav, P., Trivedi, R., Bansal, A. K. and Bhatnagar, D. (1995). Cadmium induced lipid peroxidation and status of antioxidant system in rats' tissues. *J. Trace. Elem. Med. Biol.* **9**: 144-149.

Satarug, S., Baker, J.R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P.E., Williams, D.J., Moore, M.R., 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol. Lett. 137, 65–83.

Schäfer, S.G., Dawes, R.L.F., Elsenhan, B., Forth, W., Schümann, K., 1999. Metals. In: Marquardt, H., Schäfer, S.G., McClellan, R.O. (Eds.), Toxicology. Academic Press, San Diego, CA, pp. 755–790

Schindhelm, R.K., Dekker, J.M., Nijpels, G., Stehouwer, C.D., Bouter, L.M., Heine, R.J. and Diamant, M. (2007). Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the Hoorn Study. *Diabet. Med.*, **24**: 430-435

Schindhelm, R.K., Diamant, M., Dekker, J.M., Tushuizen, M.E., Teerlink, T. and Heine, R.J. (2006). Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab. Res. Rev.*, **22**: 437-443

Schmidt, E., and Schmidt, F.W. (1963). *Enzym. Biol. Clin*., **3**: 1.

Schroeder, W.A., Shelton, J.R., Shelton, J.B., Robberson, B. and Apell, G. (1969). "The amino acid sequence of bovine liver catalase: a preliminary report". *Arch. Biochem. Biophys.* **131** (2): 653–655.

Seifried, H.E., Anderson, D.E., Fisher, E.I. and Milner, J.A. (2007).A review of the interaction among dietary antioxidants and reactive oxygen species. *Nutr.Biochem*. 18:567-579.

Sendelbach, L.E. and Klaassen, C.D. (1988). Kidney synthesizes less metallothionein than liver in response to cadmium chloride and cadmium-metallothionein. *Toxicol. Appl. Pharmacol.*, **92**:95-102.

Sethi, P.K. and Khandelwal, D.J. (2006). Cadmium exposure: health hazards of silver cottage industry in developing countries. *Med. Toxicol.* **2**: 14-5.

Shaikh, Z.A., Vu, T.T. and Zaman, K. (1999). Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. *Toxicol. Appl. Pharmacol.*, **154**: 256-263, 1999.

Shapiro, R.L., Charles, H. and David L.S. (1998). "Botulism in the United States: A Clinical and Epidemiologic Review". *Annals of Internal Medicine* **129** (3): 221–8.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental physiology*, **82**: 291-295.

Singh, P., Chaudhary, S., Patni, A. and Sankhla V. (2007). Effect of cadmium chloride induced genotoxicity in bone marrow chromosomes of Swiss albino mice and subsequent protective effects of Emblica officinalis and vitamin C. *J of Herbal Medicine and Toxicology*. **1** (2): 67-71.

Siu, Y.P., Leung, K.T., Tong, M.K., et al: (2006). Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am. J. Kidney. Dis.*, **47**:51-9.

Smith, A.H., Lingas, E.O., & Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh. Bulletin of the World Health Organization, 78(9):

Snow, M.J., Harris, M.M. (2004).On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chem*., **84**: 145-147.

Squibb, K.S., Pritchard, J.B. and Fowler, B.A. (1984). Cadmium-metallothionein nephropathy: Relationships between ultrastructural/biochemical alterations and intracellular cadmium binding. *J. Pharmacol. Exp. Therap.,* **229**:311-321.

Srinivasa, R.P.S., Yamada, Y. and Leung, K.Y. (2003). "A major catalase (KatB) that is required for resistance to H2O2 and phagocyte-mediated killing in Edwardsiella tarda". *Microbiology (Reading, Engl.)* **149** (9): 2635–2644.

Stohs, S.J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.*, **18**: 321-336.

Stohs, S.J., Bagchi, D., Hassoun, E. and Bagchi M. (2000). Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J Environ Pathol Toxicol Oncol* **19:** 201- 213.

Stowe, H.D., Wilson, M., Goyer, R.A. (1972). Clinical and morphological effects of oral cadmium toxicity in rabbits. *Arch. Pathol.*, **94**:389-405.

Stryer, L. (1999). Biochemistry. Freeman and company, San Francisco.

Sugita, M., Tsuchiya, K., 1995. Estimation of variation among individuals of biological half-time of cadmium calculated from accumulation data. Environ. Res. 68, 31–37.

Sumner, J.B. and Dounce, A.L. (1937). "Crystalline catalase". *Science* **85** (2206): 366–367.

Sumner, J.B. and Gralén, N. (1938). "The molecular weight of crystalline catalase". *Science* **87** (2256): 284–284.

Suzuki, C.A.M. and Cherian, M.G. (1987). Renal toxicity of cadmium-metallothionein and enzymuria in rats. *J. Pharmacol. Exp. Ther.*, **240**:314-319.

Swiergosz, R., Zakrzewska, M., Sawicka-Kapusta, K., Bacia, K. and Jankowska, I. (1998) Accumulation of cadmium and its effect on bank vole tissues after chronic exposure. *Ecotoxicol Environ Saf.* **41:** 130-136.

Tandon, S.K., Singh, S. and Prasad, S. (2003) Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. *Toxicol. Lett.* **145**(3):211–217.

Tanih, N.F., Dube, C., Green, E., Mkwetshana, N., Clarke, A.M., Ndip, L.M. and Ndip, R.N. (2009). An African perspective on *Helicobacter pylori*: prevalence of human infection, drug resistance, and alternative approaches to treatment. *Ann. Trop. Med. Parasitol*., **103**: 189-204.

Thimbrel, J.A. (1991). *Principles of Biochemical Toxicology.* Taylor and Francis, London, U.K

Toner, K., Sojka, G. and Ellis, R. (2000). "A Quantitative Enzyme Study; CATALASE". bucknell.edu.

Tzirogiannis, K.N., Panoutsopoulos, G.I., Demonakou, M.D., Hereti, R.I., Alexandropoulou, K.N., Basayannis, A.C. and Mykoniatis, M.G. (2003). Time-course of cadmium-induced acute hepatotoxicity in the rat liver: the role of apoptosis. *Arch. Toxicol.*, **77**: 694-701.

Umanzor, J., Aguiluz, M. and Pineda, C. (2006). Concurrent cisplatin/gemcitabine chemotherapy along with radiotherapy in locally advanced cervical carcinoma: A phase II trial. *Gynecol Oncol*, **100**: 70-5.

Vahter, M., 1999. Methylation of inorganic arsenic in different mammalian species and population groups. Sci. Prog. 82, 69–88.

Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology 181–182, 211–217.

Valko, M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160:1-40.

Vellinger C., Parant, M., Rousselle, P. and Usseglio-Polatera, P.(2012). Antagonistic toxicity of arsenate and cadmium in a freshwater amphipod (Gammarus pulex). Ecotoxicology **21**(7):1817-27.

Viaene, M.K., Masschelein, R., Leen- ders, J., De, G.M., Swerts, L.J., and Roels, H.A. (2000). Neurobehavioural effects of occupational expo- sure to cadmium: a cross-sectional epidemiological study. *Occup. Environ. Med.* 57, 19–27.

Viana, G. F. De Sousa, Garcia, K.S., and Menezes-Filho, J.A (2011) Assessment of carcinogenic heavy metal levels in Brazilian cigarettes. *Environ. Monitor. Assessment* 181(1–4):255–265

Vinodhini, R. and Narayana, M.W (2008). Effect of heavy metals Induced Toxicity on Metabolic biomarkers in common carp (cyprinus carpio l). *Mj. Int. J. Sch. Tech.* **2**(1):192-200

Vromman, V., Saegerman, C., Pussemier, L., Huyghebaert, A. De Temmerman, L., Pizzolon, J.C., and Waegeneers, N. (2008). Cadmium in the food chain near non-ferrous metal production sites *Food Additives and Contaminants*, **25**(3): 293–301

Waalkes, M.P. and Rehm S. (1994). Chronic toxic and carcinogenic effects of cadmium chloride in male DBA/2NCr and NFS/NCr mice: Strain dependent association with tumors of the hematopoietic system, injection site, liver, and lung. *Fund Appl. Toxicol.* **23**:21-31.

Waisberg, M., Joseph, P., Hale, B. and Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis: a review. *Toxicology*, **192**: 95-117, 2003.

Walker, N. and Lorimer, A.R (2004). Coronary heart disease: Epidemiology, pathology and diagnosis. In: Lindsay G, Gaw A, editors. *Coronary Heart Disease Prevention. A Handbook for the Health Care Team*. 2nd ed. London: Churchill Livingstone

Wang, B., Shao, C., Li, Y., Tan, Y. and Cai, L. (2012). Cadmium and its epigenetic effects. *Curr. Med. Chem.*, **19**(16): 2611-2620.

Wang, Z.Y (2001). Arsenic compounds as anticancer agents. Cancer Chemother Pharmacol. 2001 Aug;48 Suppl 1:S72-6.

Ware, G.W., ed. (1989) Cadmium. US Environmental Protection Agency Office of Drinking Water health advisories. *Reviews of Environmental Contamination and Toxicology*, **107**:25–37.

Wedepohl, K. H. (1995). "The composition of the continental crust". *Geochimica. et. Cosmochimica Acta*., **59** (7): 1217–1232.

WHO (1981). Environmental Health Criteria. 18: Arsenic. World Health Organisation, Geneva.

WHO (1992). *Cadmium.* Geneva, World Health Organization

Wojchiech, W.J. and Konrad, R. (2001). Blood concentration of essential trace element and heavy metals in workers exposed to lead and cadmium. *Inter. J. Occup. Med. Environ Health*, 14(3):223-229.

World Health Organization (2002). Reduced Osmolarity Oral Rehydration Salts (ORS) Formulation. World Health Organization, Geneva.

World Health Organization, (1995). Inorganic constituents and physical parameters. In: Guidelines for DrinkingWater-Quality: Health Criteria and other Supporting Information, World Health Organization, Geneva.

Wright, R.O., and Baccarelli, A. (2007). Metals and neurotoxicology. *J. Nutr.* **137**, 2809–2813.

Wu, J.T. and Wu, L.L. (2007). Chronic systemic inflammation leading eventually to myocardial infarction, stroke, COPD, renal failure and cancer is induced by multiple risk factors. *J. Biomed. Lab. Sci*., **19**:1-6.

Xu, C., Holscher, M.A. and Jones, M.M. (1995). Effect of monoisoamyl meso-2,3-dimercaptosuccinate on the pathology of acute cadmium intoxication. *J. Toxicol. Environ. Health*, **45**:261-277.

Xu, L.C., Wang, S.Y., Yang, X.F. and Wang, X.R. (2001). Effects of cadmium on rat sperm motility evaluated with computer assisted sperm analysis. *Biomed. Environ. Sci.*, **14**:312–7.

Yang, Z., Yang, S., Qian, S.Y., Hong, J.S., Kadiiska, M.B., Tennant, R.W., Waalkes, M.P., and Liu,J. (2007). Cadmium-induced toxicity in rat primary mid-brain neuroglia-cultures: role of oxidative stress from microglia. *Toxicol. Sci.* **98**, 488–494.

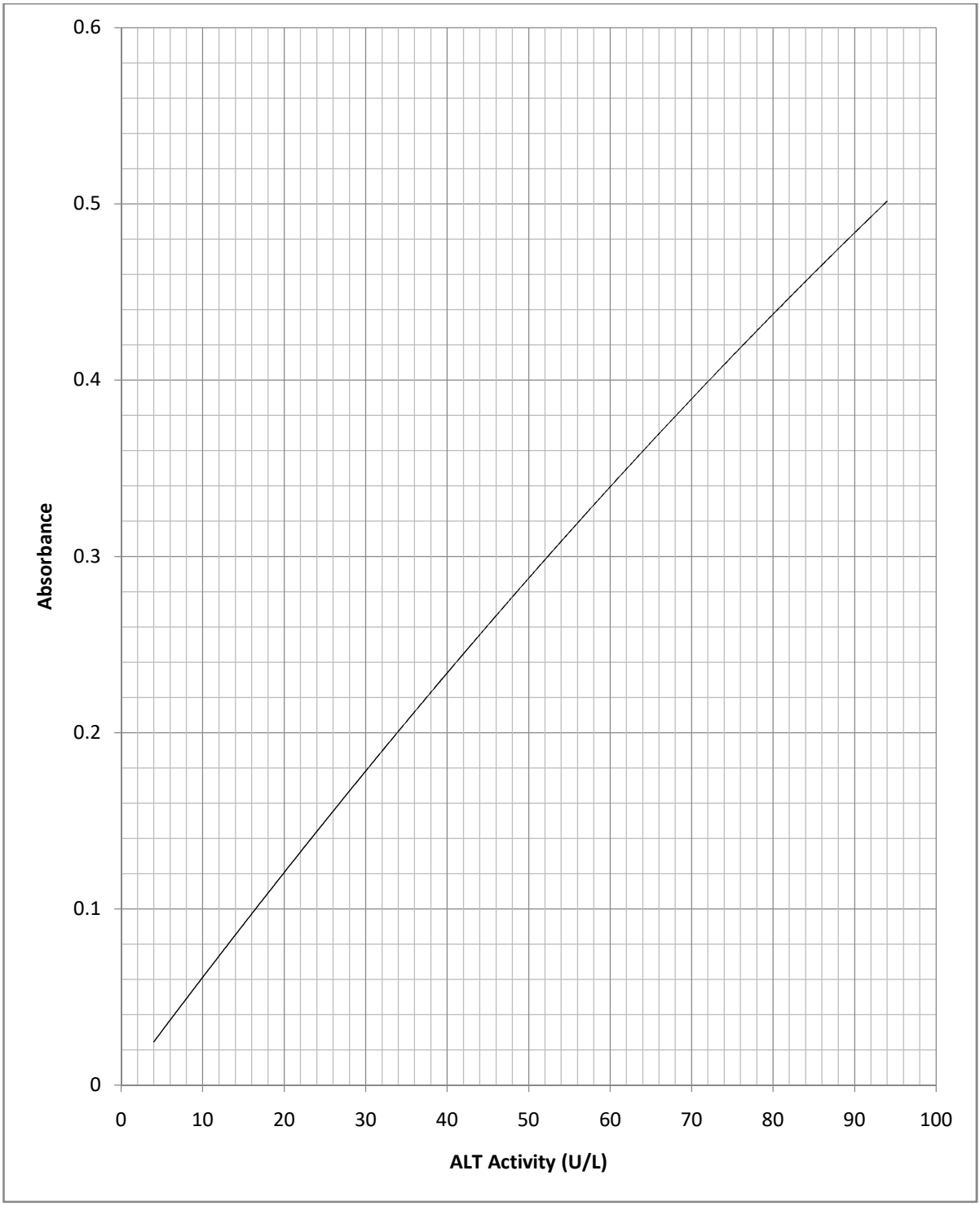
Yin, S.J., Chern, L.L., Shen, J.Y. (1999). Cadmium Induced Renal Lipid Peroxidation in Rats and Protection by Selenium. *J. Toxicol. Environ. Health* 57: 403

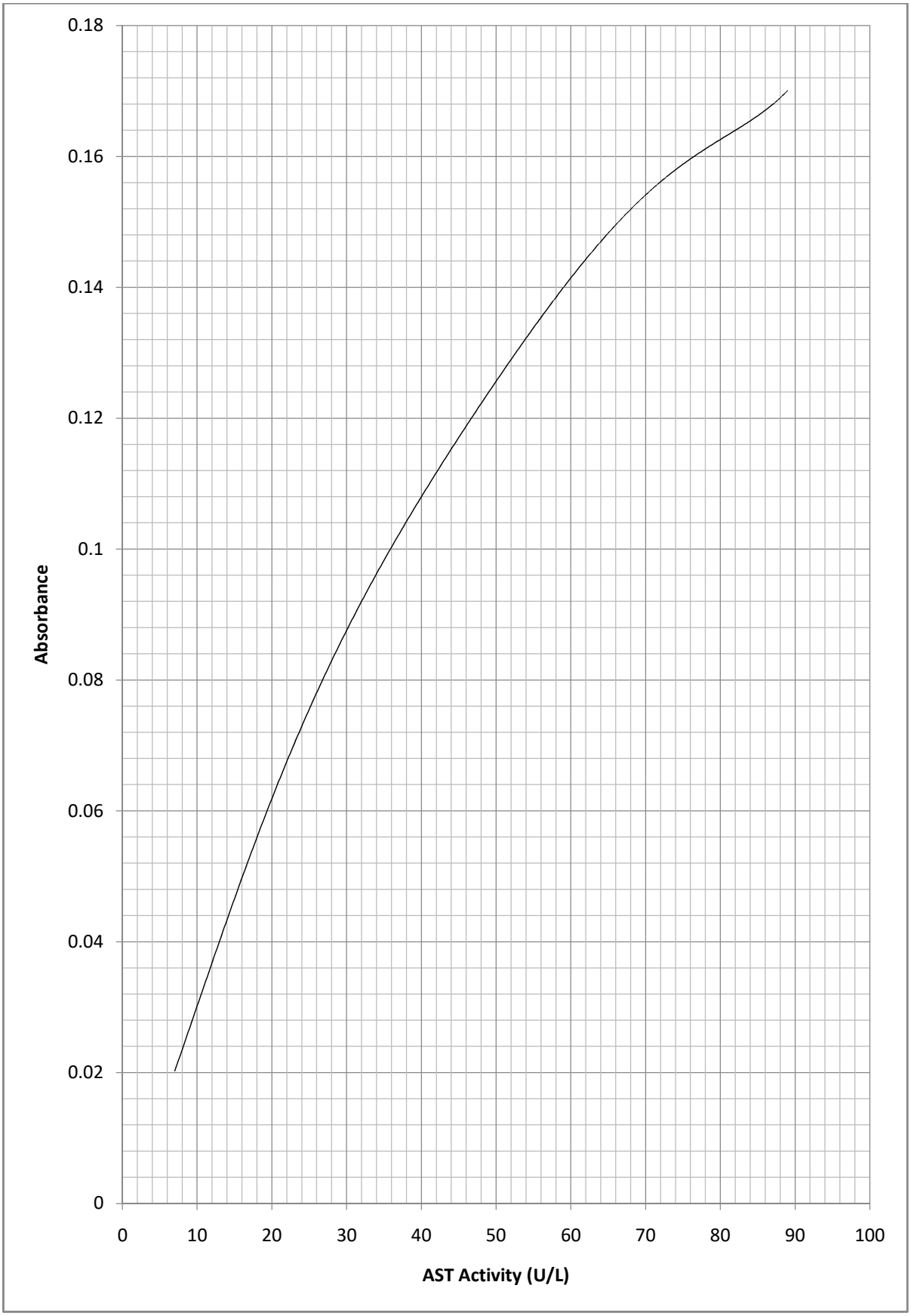
Zhao, C.Q., Young, M.R., Diwan, B.A., Coogan, T.P., Waalkes, M.P., 1997. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proc. Natl. Acad. Sci. U.S.A. 94, 10907–10912.

Žikić, R., Štajn, A., Saičić, Z., Spasić, M., Ziemnicki, K. and Petrović, V. (1996). The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (Carassius auratus gibelio Bloch.) exposed to cadmium. *Physiol Res*, **45**: 479-481.

APPENDIX I

Standard Plot for ALT and AST Activities




0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Absorbance
0
10
20
30
40
50
60
70
80
90
100
AST Activity (U/L)

APPENDIX II

FORMULA FOR STATISTICAL EVALUATION OF DATA

The values obtained from the assays were analyzed using mean and standard deviations.

The statistical formulas are given below

1. Mean (x) = $\frac{\sum x}{n}$

2. Standard deviation (δ) = $\sqrt{\frac{\sum(x-x)^2}{n-1}}$

Where ∑ = Summation

x = individual samples

n = number of sample activity

Analysis of variance was calculated in each case using the formulae below.

1. Correction Term (CT)

$$CT = \frac{(\sum\sum x)^2}{N}$$

2. Total Sum of Squares (TSS)

$$TSS = \sum\sum x^2 - CT$$

3. Treatment Sum of Squares (TrSS)

$$TrSS = \frac{\sum(Treatment\ Totals)^2}{No\ of\ replicates} - CT$$

4. Error Sum of Squares (ESS)

ESS = TSS – TrSS

5. Mean Square (MS)

$$MS = \frac{Sum\ of\ Square\ (SS)}{Degree\ of\ Freedom\ (DF)}$$

Treatment MS (TrMS) = $\frac{TrSS}{TrDF}$

Error MS (EMS) = $\frac{ESS}{EDF}$

6. Degree of Freedom

Total DF (TDF) = N -1

N = Total number of observation

Treatment DF (TrDF) = Number of Treatment – 1

Error DF (EDF) = TDF –TrDF

Anova Summary Table

Source of Variance	DF	SS	MS	F-value	F-critical
Total	TDF	TSS			
Treatment	TrDF	TrSS	TrMS	F-value	F-critical
Error	EDF	ESS	EMS		

Least significant difference (LSD) test

LSD = $(Sx_1 - x_2)t$

t = tabular or critical $t_{0.05}$, df (error)

$Sx_1 - x_2$= Standard error of difference between means

$Sx_1 - x_2 = \sqrt{\frac{2 \times EMS}{N}}$

N = number of replicates per treatment