2. REVIEW OF LITERATURE

2.1. DEFINING TOXICOLOGY

Toxicology is the study of how natural or man-made poisons (Greek, toxon, “bow” and toxicon, “arrow poison”) cause undesirable effects by reacting with specific cellular components to destroy cells, alter growth or development and thereby damaging the normal functioning of living organisms. The word “toxicity” describes the degree to which a substance is poisonous or can cause injury once it reaches a susceptible site in, or on, the body.. The toxicity depends on a variety of factors including dose, duration and route of exposure, shape and structure of the chemical.

Toxicology addresses a vast multidisciplinary area in the field of scientific research which includes Environmental Toxicology, Occupational Toxicology, Regulatory Toxicology, Food Toxicology, Clinical Toxicology, Descriptive Toxicology, Forensic Toxicology, Analytical Toxicology and Mechanistic Toxicology.

Acute poisoning is characterized by sudden and severe exposure and rapid absorption of the substance. Normally, a single large exposure is involved. Chronic poisoning is characterized by prolonged or repeated exposures of a duration measured in days, months or years. A local effect refers to an adverse health effect that takes place at the point or area of contact. Systemic effect refers to an adverse health effect that takes place at a location distant from the body’s initial point of contact. Cumulative poisons are characterized by materials that tend to build up in the body as a result of numerous chronic exposures. When two or more hazardous materials are present at the same time, the resulting effect can be greater than the effect predicted based on the additive effect of the individual substances. This is called a synergistic or potentiating effect

The route of intake and the dosage determine the intensity and duration of the harmful

effects of a toxicant. Though metal toxicity is usually experienced through oral (enteral) routes, parenteral administration provides the desired comparative data needed for comparative toxicology. In some instances, parenteral administration is essential for the drug to be administered in its active form. Availability is usually more rapid, extensive and predictable than when a drug is given by mouth. The effective dose therefore can be more accurately delivered. In clinical studies, parenteral administration is advantageous when the subjects are uncooperative, unconscious or unable to retain anything given by mouth. A common route of administration is the intraperitoneal route. Intraperitoneal injection is the administration of chemicals into the peritoneal cavity of animals for evaluation of their biologic action and toxicity. The peritoneum, a serous or mesothelial membrane lining the abdominal cavity acts as a semipermeable membrane and is connected with an efficient vascular system (Wilkinson G.R., 2001).

Soluble metal compounds that are soluble and stable at the peritoneal fluid pH (5-7) are rapidly absorbed across the visceral peritoneum and transferred to the liver via the portal circulation. Hepatic cells process absorbed material before it reaches other tissues. The microparticulates of the absorbed material are phagocytized by the invading macrophages and scavenged into the reticulo-endothelial system, which includes liver and spleen. Thus, although absorption of soluble metal salts from intraperitoneal injections is rapid, the toxicity is less due to possible detoxification by the liver (Schumann G.B. et al, 1980).

2.2. HEAVY METAL TOXICITY

Heavy metals are ubiquitous in the biosphere. Anthropogenic activities have also introduced substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sinks (Chiesa *et al*., 2006). Industrial and municipal waste waters and mine run offs cause various metals and metallic compounds to be released into water.

These pollutants also enter the water bodies as acid rain and their sulfate and nitrate ions make water acidic. Most inorganic compounds are toxic, particularly, those of heavy metals such as mercury, chromium, lead, silver, cadmium, etc., which come from paint and plastic production, metal and metal plating units etc. These substances get attached to the tissues of aquatic organisms, produce physiological poisoning and are therefore, capable of killing living organisms of water bodies. Lead has been employed in the past to manufacture of drain pipes, cookery pots, weapons and machinery (Vogiatzis and Loumbourdis, 1999). With the advent of science and technology, it has been employed in varieties of industrial applications and products such as storage batteries, chemicals, pigments, paints, gasoline (Landrigan *et al*., 1990), in mining and smelting activities. Massive lead utilization has resulted in extensive lead contamination of the environment. Arsenic has been used in medicine for centuries. Many products containing inorganic and organic arsenic were employed for the treatment of anaemia, epilepsy, asthma, psoriasis, syphilis, lichen planus and many other diseases (Alain *et al*., 1993). The element is recovered as a byproduct from the smelting of Cu, Pb, Zn and other ores (Englyst *et al*, 2001). This can result in the release of arsenic in the environment. Other sources of arsenic contamination are mining and smelting industry, electroplating and semiconductor industry as well as agriculture (Bustamante *et al*., 1997). Application of pesticides and herbicides containing arsenic has increased its environmental dispersion. They represent a significant ecological and public health concern due to their toxicity and their ability to accumulate in living organisms.

2.3. LEAD

2.3.1. Properties of lead

Elemental lead is a bluish-gray, soft metal of atomic weight 207.19 and atomic number 82 [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$. Lead occurs in four valence states: elemental (PbO), monovalent (Pb^+),

divalent (Pb^{2+}), and tetravalent (Pb^{4+}). It is a major constituent of more than 200 identified minerals, of which galena (PbS), angelesite ($PbSO_4$), cerusite ($PbCO_3$), mimetite ($PbCl_2.3Pb_3(AsO_4)_2$) and pyromorphite ($PbCl_2.3Pb_3(PO_4)_2$) are sufficiently abundant to form mineral deposits (EPA, 1980); galena being the primary form of lead in the natural state.

2.3.2. Sources and uses of lead

Lead and its compounds have been known to man for about 7,000 years, and lead poisoning has occurred for at least 2,500 years (Barth *et al*., 1973). In Egypt, between 5,000 and 7,000 BC, lead was used for glazing pottery, solder, ornaments, net sinker, anchors, caulking, coins, weights, aqueducts, piping, and cooking utensils (Nriagu, 1978a). Lead acetate was used as a sweetener for wine and cider as well as in medicine for treating several diseases. The biocidal properties of lead were familiar to the ancient Egyptians, and lead salts were sometimes used by them for homicidal purposes (De Michele, 1984). In the late 1700's, symptoms of acute lead poisoning recorded among industrial workers were called "Mill Reek" or "Devonshire Colic" (NRCC, 1973). The decline of the Roman Empire may have been hastened by endemic lead poisoning--a theory supported by residue data showing high lead concentrations in bones and remains of Roman aristocrats (Nriagu, 1978a)--perhaps through ingestion of excessive amounts of wine laced with lead (De Michele, 1984). Lead is also a common contaminant of illicitly distilled whiskey in which automobile radiators are frequently used as condensers. Herbal remedies from the Indian subcontinent have been found to have high concentrations of heavy metals and unsupervised treatment may result in toxicity.

2.3.3. Distribution of lead in the environment

Lead enters the atmosphere mainly through smelter emissions, primarily as $PbSO_4$ and PbO-$PbSO_4$, and through vehicle emissions, which include unburned lead, tetraethyl lead (TEL), tetramethyl lead (TML), and various lead halides, sulfates, phosphates, and oxides (Harrison

and Laxen, 1981). Anthropogenic activities leading to increased air lead levels include primary and secondary lead smelting, the burning of gasoline containing lead antiknock agents, coal combustion, storage battery manufacture, and pigment production (NRCC, 1973). Lead reaches the aquatic environment through industrial and municipal discharges, in atmospheric deposition, from weathering processes in areas of natural lead mineralization, and in highway runoff (EPA,1980; Harrison and Laxen, 1981; Birdsall *et al*., 1986). Industrial lead input to aquatic environments is estimated at 10X that introduced by natural weathering processes (Scoullos, 1986).

2.3.4. Mode of action of lead

Lead modifies the function and structure of kidney, bone, the central nervous system, and the hematopoietic system and produces adverse biochemical, histopathological, neuropsychological, foetotoxic, teratogenic, and reproductive effects (Boggess, 1977; Nriagu, 1978b; De Michele, 1984).

Lead interferes with a variety of cell functions, including cell membrane integrity, neurotransmitter function, heme synthesis and mitochondrial oxidative phosphorylation. In the case of heme synthesis, for example, lead inhibits enzymes in the porphyrin pathway resulting in increases of substrates, which are subsequently eliminated and can be measured in urine. There is also inhibition of the ferrochelatase enzyme, resulting in failure to incorporate iron into the tetrapyrrole ring of heme (Yang and Lewandrowski, 2002).

2.3.5. Toxicokinetics of lead

a) Absorption

Inorganic lead is rapidly absorbed in gastrointestinal tract upon inhalation and ingestion. Alkyl lead compounds (methyl and tetraethyl lead) readily undergo dermal absorption

because of its lipid solubility. Absorbed lead is rapidly taken up by blood and soft tissue, and then slowly redistributed to bone. Bone accumulates lead during much of the lifespan and may then serve as an endogenous source, releasing lead slowly back into the blood after the exposure stops (IPCS, 1995).

b) Distribution

Lead is distributed to soft tissues (kidney and liver); bone, teeth and hair mostly as a phosphate salt. Rates of absorption and distribution are greatly influenced by dietary intake and body stores of phosphate, calcium and iron relative to lead (De Michele, 1984).

c) Metabolism

Inorganic lead is not known to be metabolized, but it does form complexes with a variety of protein and non-protein ligands. Lead present in blood is almost exclusively found in the erythrocytes, half of it bound to hemoglobin. Most of the total body burden of lead is found in the bones that can be mobilized to blood thus being once again bioavailable (De Michele, 1984).

d) Elimination

Bile is an important route of excretion; ingested lead probably proceeds sequentially from gut, to blood, to bone and soft tissue, and by way of the bile to small intestine and fecal excretion (De Michele, 1984).

2.3.6. Effect of lead in different organ systems

The most sensitive target of lead poisoning is the nervous system. In children, neurologic deficits have been documented at exposure levels once thought to cause no harmful effects. Exposure to lead can have a wide range of effects on a child's development and behavior. Even when exposed to small amounts of lead levels, children may appear inattentive, hyperactive and irritable. Children with greater lead levels may also have problems with

learning and reading, delayed growth and hearing loss. At high levels, lead can cause permanent brain damage and even death.

Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is sensitive to lead. A decrease in the activity of this enzyme results in an increase of the hormone, erythropoietin (EP), in the red blood cells. Lead can induce two types of anemia. Acute high-level lead poisoning has been associated with hemolytic anemia. In chronic lead poisoning, lead induces anemia by diminishing red blood cell survival (ATSDR, 1992).

Lead toxicity has endocrine effects. A strong inverse correlation exists between blood lead levels and levels of vitamin D. Because the vitamin D-endocrine system is responsible in a large part for the maintenance of extra-and intra-cellular calcium homeostasis, it is likely that lead impairs cell growth and maturation and tooth and bone development.

Long-term lead exposure has a direct nephropathy effect on the kidney. Impairment of proximal tubular function manifests a fanconi-like syndrome. There is also evidence of an association between lead exposure and hypertension, an effect that may be mediated through renal mechanisms. Gout may develop as a result of lead-induced hyperuricemia, with selective decreases in the fractional excretion of uric acid before a decline in creatine clearance. Renal failure accounts for 10% of deaths in patients with gout (ATSDR, 1992).

An increased frequently of miscarriages and stillbirths among women working in the lead trades is reported. Increasing evidence indicates that lead not only affects viability of fetus, but development as well. Developmental consequences of prenatal exposure to low levels of lead include reduced birth weight and premature birth. However, most studies in humans have failed to show a relationship between lead levels and congenital malformations.

2.4. ARSENIC

2.4.1. Properties of arsenic

Elemental arsenic (As) is a member of Group 15 of the periodic table with an atomic weight of 74.92 $[Ar]3d^{10}4s^{2}4p^{3}$ and atomic number 33. Arsenic is a metalloid that can exist in four valency states; –3, 0, +3, and +5. Arsenic exists in three allotropic forms:

1. α-arsenic or yellow arsenic, an unstable compound that rapidly forms arsenic vapour, As_4.

2. β- arsenic or black arsenic is more stable than yellow arsenic.

3. γ- arsenic or grey arsenic, the stable and common form.

Common compounds of arsenic are As(III) oxide, As(III) chloride, As(III) sulphide, As(V) oxide, arsine, arsenates.

2.4.2. Sources and uses of arsenic

Arsenic is found naturally in the environment. Historical records show that arsenic was used by the ancient Greeks, Persians, Romans, and Chinese. Arsenic has been used medicinally for more than 3000 years in Greece and Rome to cure or treat certain ailments like anemia, epilepsy, asthma, psoriasis, syphilis, lichen planus and many other diseases (Alain *et al*., 1993). Arsenic was used more than 2400 years ago in Greece and Rome as a therapeutic agent and as a poison. Composition analysis of antique statues and utensils of ancient Greece have revealed the presence of toxic metals like arsenic and lead. In Greek mythology, Hephastus, the god of smiths and Horus, the god of youth were known to have clubfeet and limp- both symptoms of arseno-neuritis. That the limp could be attributed to heavy metal toxicity was proved from the composition analysis of samples from antique statues and

utensils. It is now suggested that in ancient Greece, introduction of new smelting techniques may have exposed the metal workers to the effects of various toxic metals causing for instance, chronic lead poisoning or chronic arsenic poisoning causing peripheral neuritis with weakness or lameness of one or both lower extremities. This may explain the depiction of these gods with club-footed limps. The history and folklore of arsenic prompted extensive studies by early pharmacologists. Indeed, the foundation of many modern concepts of chemotherapy derives from Ehrlich's early work with organic arsenicals, and such drugs were once a mainstay of chemotherapy. In recent times Arsenic sprays or gases were used during World War I trench warfare, the one favored by the British was known as Lewisite. The last century saw extensive use of arsenic as a chemotherapeutic agent for acute promyelocytic leukemia before its use was banned due to toxicity. It is selectively used in homeopathic treatments.

Documented causes of arsenical poisoning have included traditional Chinese herbal balls (Espinoza and Mann, 1995), Korean herbal medicine prescribed for haemorrhoids, kelp supplements, contact with pesticides and rodenticides, industrial waste contaminations in water streams, beer, milk, rice, arsenic-treated pressurised lumber, cocaine, sinister forensic events (Gyorgyey, 1987), and even cot deaths. Cot deaths have been attributed to bio-deterioration of cot mattresses from extracellular enzymes of *Streptococcus brevicaulis* fungi converting preservative plasticisers and fire retardants to arsine and phosphines. The compound As_2O_3 has been used for the treatment of acute promyelocytic leukaemia (Soignet *et al.*, 1998). In recent times, arsenic pentoxide and As_2O_3 are used as additives in alloys, particularly with lead and copper; arsenic and As_2O_3 are used in the manufacturing of low-melting glasses. High-purity arsenic metal and gallium arsenide are used in semiconductor products. Fowler's solution (1% potassium arsenite solution) was used as a medication (Cuzick *et al.*, 1982). From the US Civil War (1861-1865) to 1910 arsenic has been the main

ingredient in embalming fluids. From the late 1800s to the 1960s it was heavily used in pesticides; indeed, pesticides accounted for the greatest use of arsenic for the first half of the twentieth century (Robinson and Ayotte, 2006). Arsenic sprays or gases were used during World War I trench warfare, the one favored by the British was known as Lewisite.

2.4.3. Distribution of arsenic in the environment

Arsenic is widely distributed in the Earth's crust, which contains about 3.4 ppm arsenic (Wedepohl, 1991). It is mostly found in nature in minerals, such as realgar (As_4S_4), orpiment (As_2S_3), and arsenolite (As_2O_3), and only found in its elemental form to a small extent. Arsenic is the main constituent of more than 200 mineral species of which about 60% is arsenate, 20% sulfide and sulfosalts and the remaining 20% include arsenides, arsenites, oxides and elemental arsenic (Onishi, 1969). In aquatic systems, inorganic arsenic occurs primarily in two oxidation states, As(V) and As(III). Both forms generally co-exist, although As (V) predominates under oxidizing conditions and As(III) predominates under reducing conditions. The most common of the arsenic minerals is arsenopyrite, FeAsS. Anthropogenic sources of arsenic releases to water include mining, nonferrous metals, especially copper, smelting, waste water, dumping of sewage sludge, coal burning power plants, manufacturing processes, urban runoff, atmospheric deposition and poultry farms (Garbarino *et al*., 2003; Nriagu and Pacyna, 1988).Smelting of non-ferrous metals and the production of energy from fossil fuel are the two major industrial processes that lead to anthropogenic arsenic contamination of air, water and soil. Other sources of contamination are the manufacture and use of arsenical pesticides and wood preservatives.

2.4.4. Mode of action of arsenic

All arsenic compounds are poisonous. In general, inorganic arsenicals are more toxic than organoarsenicals and arsenite is more toxic than arsenate. The primary mechanism of arsenite

toxicity is considered to result from its binding to protein sulfhydryl groups. Arsenate is known to affect oxidative phosphorylation by competition with phosphate. Arsenate As (V) behaves very much like phosphate consequently, it can substitute for phosphate in normal cell reactions, interesting with normal cell functions (Abernathy and Ohanian, 1993; NAS, 1977). Pentavalent arsenic uncouples oxidative phosphorylation by arsenolysis. In contrast, arsenite [As(III)] has a high affinity for thiol (-SH) groups in proteins, causing inactivation of a variety of enzymes (Abernathy and Ohanian, 1993; NAS, 1977; Tseng *et al*., 1968). Trivalent arsenic inhibits the reduction of nicotinamide adenine dinucleotide by deactivating critical enzymes in the tricarboxylic acid cycle.

2.4.5. Toxicokinetics of arsenic

a) Absorption

The major routes of arsenic absorption are ingestion and inhalation. Both pentavalent and trivalent soluble arsenic compounds ingested are rapidly and extensively absorbed from the gastrointestinal tract. The extent of absorption of inhaled arsenic depends upon its chemical form, particle size and solubility.

b) Distribution

Blood is the main vehicle for the transport of arsenic following absorption. Arsenic is rapidly distributed throughout the body including muscles, bones, kidneys, lungs, skin and excretory/storage organs, such as nails and hair, having the highest concentrations (Yamauchi and Yamamura, 1985).

c) Metabolism

In many species arsenic metabolism is characterized by two main types of reactions: (1) reduction reactions of pentavalent to trivalent arsenic, and (2) oxidative methylation reactions

in which trivalent forms of arsenic are sequentially methylated to form mono-, di- and trimethylated products using S-adenosyl methionine (SAM) as the methyl donor and glutathione (GSH) as an essential co-factor.(Styblo *et al.*, 1995; Vahter, 1995)

d) Elimination

Arsenic is eliminated by many routes- faeces, urine, sweat, milk, hair, skin and lungs (Yamauchi H and Yamamura Y, 1985). Urine is the primary route of elimination for both pentavalent and trivalent inorganic arsenicals.

2.4.6. Effects of arsenic in different organ systems

Gastrointestinal effects are seen acutely after arsenic ingestion. Extensive inflammation and necrosis of the mucosa and submucosa of the stomach and intestine may occur and progress to perforation of the gut wall. Acute arsenic toxicity may be associated with hepatic necrosis and elevated levels of liver enzymes. Chronic arsenic ingestion may lead to cirrhotic portal hypertension (ATSDR, 2007; Datta, 1976). Case reports have also linked chronic high level arsenic exposure with hepatic angiosarcoma, a rare form of liver cancer (Popper *et al*., 1978; Zaldivar *et al*., 1981; ATSDR, 2007).

The systemic toxicity occurring in severe acute arsenic poisoning may include acute tubular necrosis with acute renal failure. Chronic renal insufficiency from cortical necrosis has also been reported. The precipitating cause of renal injury may be hypotensive shock, hemoglobinuric or myoglobinuric tubular injury, or direct effects of arsenic on tubule cells. Glomerular damage can result in proteinuria. Arsine gas is more nephrotoxic than arsenic. However, both can cause acute tubular necrosis (Giberson *et al*., 1976).

Both acute and chronic exposure to high levels of arsenic may result in a wide range of adverse cardiovascular effects. Acute arsenic poisoning may cause both diffuse capillary

leakage and cardiomyopathy, resulting in shock. Arsenic ingestion from contaminated beer has been reported to cause outbreaks of cardiomyopathy (Reynolds, 1901; Aposhian, 1989; Rosenman, 2007). Other reports of arsenic poisoning have resulted in peripheral vascular disease (Engel *et al*., 1994). Inhibition of endothelial nitric oxide synthase, changes in coagulation and inflammation due to arsenic exposure have been shown in experimental studies to contribute to atherosclerosis (Simeonova and Luster, 2004).

Arsenic may cause severe neurologic effects leading to encephalopathy at acute high doses (> 2mg As/kg/day) (Uede and Furukawa, 2003; Vantroyen *et al*., 2004; ATSDR, 2007). Arsenic poisoning can cause peripheral neuropathy (Chakraborti *et al*., 2003a, 2003b).

Pigment changes and palmoplantar hyperkeratoses are characteristic of chronic arsenic exposure. Benign arsenical keratoses may progress to malignancy. Delayed effects of acute or chronic exposure may be seen as Mee's lines in nails. Mees lines are horizontal lines in the nails of digits (ATSDR, 2007).

Inhalation of high concentrations of arsenic compounds produces irritation of the respiratory mucosa. Smelter workers experiencing prolonged exposures to high concentrations of airborne arsenic had inflammatory and erosive lesions of the respiratory mucosa, including nasal septum perforation. Lung cancer has been associated with chronic arsenic exposure in smelter workers and pesticide workers (ATSDR, 2007).

Both acute and chronic arsenic poisoning may affect the hematopoietic system. A reversible bone marrow depression with pancytopenia may occur. Anemia and leukopenia are common in chronic arsenic toxicity and are often accompanied by thrombocytopenia and mild eosinophilia. There is a suggestive association between chronic arsenic exposure and immunosuppression (NRC, 2000; IARC, 2004).Basal cell carcinomas upon chronic arsenic exposure have also been reported (Cohen and Moore, 2007).

Increased frequency of spontaneous abortions and congenital malformations has been linked to arsenic exposure. Arsenic is a reproductive toxicant and a teratogen (Shalat, 1996) and is readily transferred across the placenta.

2.5. A COMPARATIVE STUDY OF LEAD AND ARSENIC TOXICITY IN DIFFERENT SPECIES

Ezemonye and Enuneku (2005) evaluated acute toxicity of lead to amphibian tadpoles (Toad: *Bufo maculatus* and frog: *Ptychadena bibroni*) and found increased mortality with increased concentration and exposure time. The clinical signs of lead toxicity in dogs include convulsions or fits, vomiting and diarrhea, abdominal pain and bizarre behaviour such as hysteria (Maddison and Hawke, 1993). Chronic lead exposure in cats can lead to impaired hemoglobin synthesis and increased red blood cell fragility, resulting in a microcytic, hypochromic anemia (Alstine *et al*., 1993). A lead poisoned bird can suffer neurological and physical effects such as weight loss and emaciation, weakness and lethargy, poor growth and development, blindness, seizures, fewer eggs laid and higher egg mortality.

Khangarot *et al*. (1985) determined the acute toxicity of arsenic in tadpoles (*Rana hexadactyla*) and found that a concentration of 249 µg As /L would kill 50 specimens in 4 days (96-h LC50). There are great differences in tolerance to arsenic among bird species. Female mallard ducklings showed a reduced growth rate when they were fed 30 mg As (V)/kg b.w. over 10 weeks (Camardese *et al*., 1990). Brown-headed cowbird (*Molothrus ater*) showed 50 percent mortality in 11 days when fed with copper acetoarsenite at 99.8 mg/kg b.w. (Eisler, 1988). Some studies indicate that arsenic is extremely toxic to avian eggs (Birge and Roberts, 1976; Gilani and Alibhai, 1990).

2.6. HEAVY METALS (LEAD AND ARSENIC) AND OXIDATIVE STRESS

Heavy metals are pervasive environmental toxicants that have been shown to exert oxidative stress on living systems through the production of reactive oxygen species (ROS), which overwhelm the cell's capacity to maintain a reduced state (Ercal *et al*., 2001; Stohs and Bagchi, 1995). Metal-induced ROS cause damage to cellular proteins, nucleic acids and lipids, leading to a variety of cellular dysfunctions including cell death. Oxidative stress occurs when generation of free radicals (i.e. substances with one or more unpaired electrons) exceed the capacity of antioxidant defense mechanisms (that is, pathways that provide protection against harmful effect of free radicals).

Lead induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning (Xu *et al*., 2008). Reactive oxygen species (ROS) generated as a result of lead exposure has been identified in liver, kidney, brain, lung, endothelial tissue, testes and sperm. Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (Sharma *et al*., 2011b; Sharma *et al*., 2011c; Sharma *et al*., 2011).

Arsenic is known to generate free radicals that are highly reactive chemically, induces oxidative stress- mediated toxicity, thus playing a fundamental role in normal physiology and in pathologic manifestations. Arsenic generates ROS and free radicals like hydrogen peroxide (H_2O_2) (Wang *et al*., 1996; Barchowsky *et al*., 1996; Chen *et al*., 1998), hydroxyl radicals species (HO^-), nitric oxide (NO^-), (Gurr *et al*., 1998) or superoxide anion (O_2^-) (Lynn *et al*., 2000), dimethyl arsinic peroxyl radical [$(CH_3)_2$ $AsOO^-$] and dimethyl arsenic radical

[$(CH_3)_2As^-$] (Yamanaka *et al*., 1997;Yamanaka *et al*., 2001). Studies have also indicated that arsenic exerts its toxicity by generating intracellular lipid peroxides (Ramos *et al*., 1995).

2.7. LEAD AND ARSENIC AS POTENT TOXICANTS AND THEIR EFFECTS ON ANTIOXIDANT SYSTEM AND IMMUNE DEFENSES

2.7.1. Effect of lead exposure

Usually the deleterious effects of oxidative stress are counteracted by endogenous antioxidant enzymes, mainly superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (Winterbourn, 1993). SOD and catalase are considered primary enzymes since they are involved in direct elimination of ROS. SOD plays an important role in protecting the cells against the toxic effects of O_2^- by catalyzing its dismutation reactions. Various findings demonstrated that lead has inhibitory effects on SOD and CAT leading to altered enzyme activity (Soltanianejad *et al*., 2003). Several studies reported alterations in antioxidant enzyme activities such as SOD, catalase and glutathione peroxidase (GPX) and changes in the concentrations of some non-enzymatic antioxidant molecules, such as glutathione (GSH) in lead exposed animals (McGowan *et al*., 1986) and workers (Sugawara *et al*., 1991; Solliway *et al*., 1996; Gayathri *et al*., 2007; Mohammad *et al*., 2008).

Lead has been revealed to have inhibitory effect on phagocytic activity of fish macrophages and thus have an inhibitory effect on cell mediated immune response (Aboud, 2010). Exposure to heavy metals like lead alters immunological competence of fish (Zelikoff, 1993). Lead exposure leads to suppression of humoral and cell mediated immune response in *Oreochromis niloticus* (Aboud, 2010). Dietert and Piepenbrink (2006) illustrated the capacity of lead to impair immune functions and dramatically shift immune functional capacity thereby reducing host defenses against infectious agents and cancer.

2.7.2. Effect of arsenic exposure

Arsenic has been reported to activate NADPH oxidase significantly and induce the production of $O_2^-$and H_2O_2 (Chen *et al*., 1998). Arsenite treatment in gold fish leads to a significant altered activity of superoxide dismutase, catalase and glutathione peroxidase (Rooney, 2007). Head kidney macrophages from exposed fish demonstrated significant levels of superoxide anions (Datta *et al*., 2009). Prolonged exposure to arsenic results in overproduction or accumulation of ROS due to dysfunction of antioxidant enzymes and overwhelms the intrinsic antioxidant defenses of the head kidney macrophage towards pathological situation (Gwinn and Vallyathan, 2006).

Phagocytic activity of macrophages and other immune responses were found to be significantly reduced by arsenic exposure in birds (Fairbrother *et al*., 1994; Vodela *et al*., 1997). Generally, arsenic can disrupt glucocorticoid regulation of immune function (Kaltreider *et al*., 2001) and arsenic-mediated apoptosis may lead to a diminished immune response in mice (Harrison and McCoy, 2001), rats (Bustamante *et al*., 1997) and humans (de la Fuente *et al*., 2002; Gonzales- Rangel *et al*., 2005). Additionally, arsenic exposure in mice has been shown to suppress the primary antibody response (Sikorski *et al*., 1991), reduce macrophage and neutrophil abundance (Patterson *et al*., 2004), increase susceptibility to infection (Aranyi *et al*., 1985), increase mortality due to bacterial infection (Hatch *et al*., 1985), decrease adhesion of macrophages, decrease nitric oxide (NO) production, and reduce chemotactic and phagocytotic indices (Sengupta and Bishayi, 2002; Bishayi and Sengupta, 2003).

2.7.3. Molecular basis of heavy metal induced toxicity on the dual defenses in the body

a) Antioxidant defenses

Heavy metals have electron- sharing affinity that can result in formation of covalent attachments (Bondy, 1996). These attachments are mainly formed between heavy metals and

sulfhydryl groups of proteins. Several enzymes in antioxidant defense system become inactive or non-functional due to direct binding of the metal to the enzymes' active sites, if the sites contain sulfhydryl groups (Quig, 1998). Interaction of toxic metals with reduced glutathione (GSH) metabolism is an essential part of the toxic response of many heavy metals (Hultberg *et al*., 2001). The proposed mechanism for metal- induced oxidative stress is often explained by addressing their role in the generation of reactive oxygen species (ROS) and their effect on the antioxidant defense system.

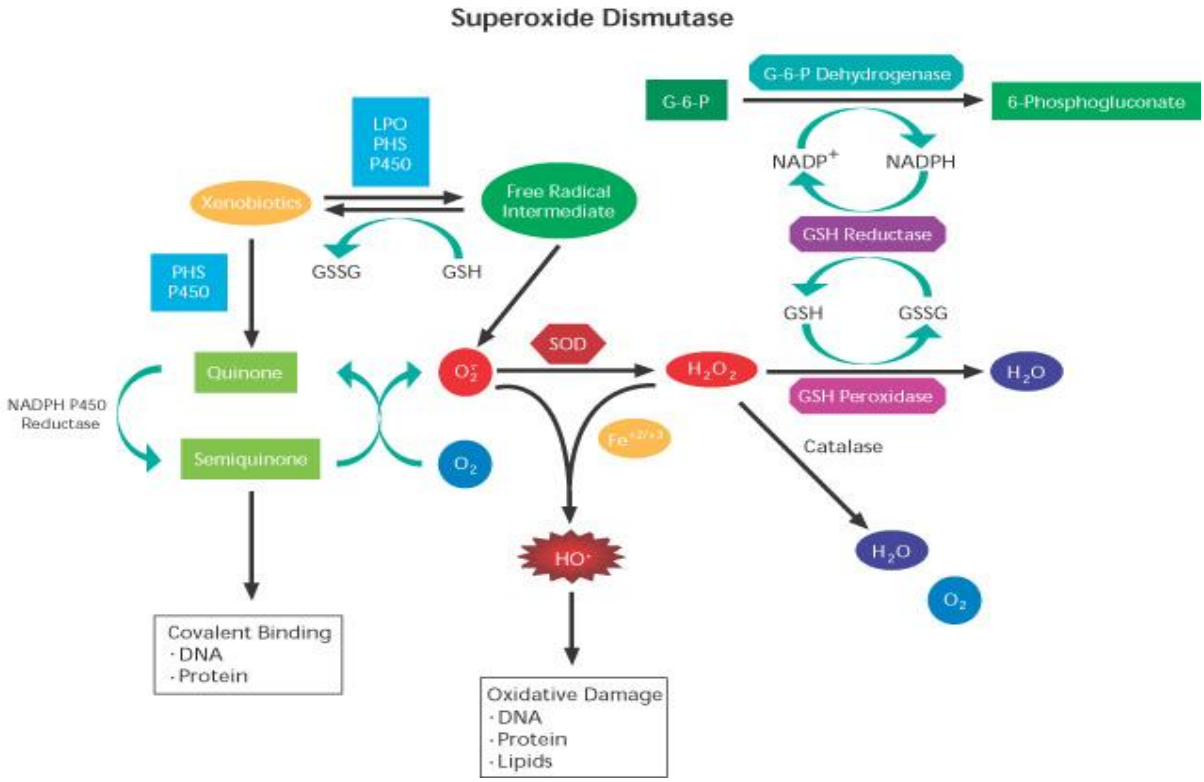

Fig I: Relationship between antioxidant defense mechanisms and oxidative stress in living organisms.

Oxidative stress is imposed on cells as a result of one of three factors: 1) an increase in oxidant generation, 2) a decrease in antioxidant protection, or 3) a failure to repair oxidative damage. Cell damage is induced by reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them. Examples are

hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. The main source of ROS *in vivo* is aerobic respiration, although ROS are also produced by peroxisomal b-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. Under normal conditions, ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA (Fiers, 1999; Nicholls and Budd, 2000; Hayes, 1999).

Antioxidant enzymes which remove peroxides, and superoxide radicals including glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD), are potential targets for heavy metals like lead, arsenic, cadmium and mercury. Because GPx requires selenium for its activity, when lead forms a complex with selenium, GPx activity decreases (Howard, 1974; Whanger, 1992). Lead is known to inhibit heme synthesis, and since catalase is a heme-containing enzyme, it leads to decrease in catalase activity (Mylroie *et al*., 1984). One of the most important mechanisms for mercury induced oxidative damage is its sulfhydryl reactivity. Hg^{2+} and MeHg form covalent bonds with GSH and cysteine residues of proteins (Quig, 1998). Inorganic mercury is suggested to increase H_2O_2 production by impairing the efficiency of oxidative phosphorylation and electron transport chain (Lund *et al*., 1991; Chavez and Holguin, 1988; Nath *et al*., 1996). Arsenic toxicity is postulated to be primarily due to the binding of arsenic (III) to sulfhydryl group containing enzymes. Trivalent arsenic toxicity could be carried out either directly by attacking –SH groups, or indirectly through generation of reactive oxygen species (ROS) (Chen *et al*., 1998). Dimethylarsine, a trivalent arsenic form, can react with molecular oxygen form a $(CH_3)_2As.$ radicals and superoxide anions. This $(CH_3)_2As$ can add another molecule of molecular

oxygen and form the $(CH_3)_2$ AsO^- radical that can cause oxidative stress leading to a compromised antioxidant defense system. Arsenic also reduces antioxidant levels in plasma, which may accelerate disease development at target site. It has been postulated that cadmium too overwhelms the defense system by challenging the thiol status of cells. Cadmium induced disturbances in GSH and metallothionein levels allow free radicals to attack double bonds in membrane lipids and result in an increase in lipid peroxidation.

b) Innate immune response

All species require a rapid, systemic reply to foreign particles in their environment. This response is known as the innate immune response and is characterized by *de novo* synthesis of mediators that directly or indirectly through phagocytosis remove and kill the pathogen (Ulevitch, 2000). The immediate, innate response is mediated largely by white blood cells such as neutrophils and macrophages, cells that phagocytose and kill the foreign body, and that concurrently coordinate additional host responses by synthesizing a wide range of inflammatory mediators and cytokines which reflect the battle between the host and the invading antigen. In macrophages, the infectious agent is killed and degraded within the maturing phagosome, and components of the antigen are presented to T cells, resulting in the activation of the adaptive immune response and the establishment of protective immunity (Aderem and Underhill, 1999). A primary challenge to the innate immune system is the discrimination of a large number of potential pathogens from self, with the use of a restricted number of receptors. This challenge has been met by the evolution of a variety of receptors that recognize conserved motifs on pathogens that are not found in higher eukaryotes. These motifs have essential roles in the biology of the invading agents, and are therefore not subject to high mutation rates. Janeway and Medzhitov (1998) have provided a set of definitions to formalize a description of the components of the innate immune system. They propose calling the motifs pathogen associated molecular patterns (PAMPs), and their cognate binding

partners on the phagocytes pattern-recognition receptors. The innate immune system provides protection, in part, due to the synthesis of potent antimicrobial peptides. These peptides are induced in response to signalling pathways activated by members of the TLR family. During phagocytosis, TLRs are recruited to the phagosomes, where they sample the contents and determine the nature of the antigen (Underhill *et al*., 1999). Thus specific TLRs might distinguish between components in the phagosome and participate in the formulation of an inflammatory response appropriate for defense against a specific pathogen.