CHAPTER - 2

INTRODUCTION AND AIM OF THE STUDY

2. INTRODUCTION

2.1 Oxidative stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA.

In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, Heart failure, Myocardial Infarction, Alzheimer's disease and chronic fatigue syndrome.

Oxidative stress is in other words a large decrease in the reducing capacity of the cellular redox couples, such as glutathione (Schafer and Buettner, 2001).

Severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon et al., 1991).

The destructive aspect of oxidative stress is the production of reactive oxygen species, this includes free radicals and peroxides.ROS include oxygen-based free radicals, e.g.superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), alkoxyl ($RO^{\cdot}$), peroxyl ($ROO^{\cdot}$) and hydroperoxyl ($ROOH^{\cdot}$). Other ROS (e.g. hydrogen peroxide and lipid peroxides) can be converted into free radicals by transition metals, either free in the cell or protein-bound. Some of the less reactive of these species(such as superoxide)can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage(Valko et al.,2005).The damage these oxygen-derived species cause to cells is constantly repaired.However,under the severe levels of oxidative stress that cause necrosis, the damage cause ATP depletion, preventing apoptotic death and causing the cell to simply fall apart(Lelli et al.,1998;Lee and Shacter, 1999).

The most important source of reactive oxygen under normal conditions in humans may be the leakage of activated oxygen from mitochondria during oxidative phosphorylation.

Enzymes capable of producing superoxide are xanthine oxidase, NADPH oxidases and cytochromes P450. Hydrogen peroxide is produced by a wide variety of enzymes including several oxidases.Reactive oxygen species also plays important roles in cell signaling, a process termed redox signaling.Thus, to maintain proper cellular homeostasis, a balance must be struck between reactive oxygen production and consumption.

Metals such as iron, copper, chromium, vanadium and cobalt are capable of redox cycling in which a single electron may be accepted or donated by the metal. This action catalyzes reactions that produce reactive radicals and can produce reactive oxygen species. The hydroxyl radical can lead to modifications of amino acids, carbohydrates, initiate lipid peroxidation, and oxidize nucleobases.Most enzymes that produce reactive oxygen species contain one of these metals. The presence of such metals in biological systems in an uncomplexed form (not in a protein or other protective metal complex) can significantly increase the level of oxidative stress. In humans, hemochromatosis is associated with increased tissue iron levels, Wilson's disease with increased tissue levels of copper and chronic manganism with exposure to manganese ores.

Certain organic compounds like quinones in addition to metal redox catalyst can also produce reactive oxygen species.Quinones can redox cycle with their conjugate semiquinones and hydroquinones, in some cases catalyzing the production of superoxide from dioxygen or hydrogen peroxide from superoxide.

The majority of free radicals produced in vivo are oxidants, which are capable of oxidizing a range of biological molecules, including carbohydrates, amino acids, fatty acids and nucleotides. As it is impossible to prevent all free radical production in vivo, it is not surprising that a range of antioxidant defences have evolved in the body. Both enzymic and non-enzymic antioxidants are present. Antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. The main non-enzymic antioxidants antioxidants include GSH, vitamin C and vitamin E.The antioxidant defences of the body are usually adequate to prevent substantial tissue damage. However, an overproduction of free radicals or a drop in

the level of the antioxidant defences will lead to an imbalance and cause deleterious effects, a situation known as oxidative stress.

2.1.2 Measurement of Oxidative Stress

All multicell organisms produce energy from the oxidation of molecules by oxygen-i.e., by respiration. However, oxygen is converted by our cells to toxic metabolites such as superoxide, singlet oxygen, hydrogen peroxide, and organic oxy-radicals (McCord and Fridovich, 1978).Cells of all plants and animals show a continuous level of oxidative damage (Halliwell and Gutteridge, 1989; Pryor and Godber, 1991). Protective mechanisms against this damage and its repair are very important to the cell.

A number of both exogenous and endogenous factors can increase the oxidative stress on humans which includes disease, trauma, and exposure to toxins, exercise, perhaps mental stress, and probably aging itself. The hypothesis suggested here is that oxidative stress increases in persons with certain diseases and that the measurement of an individual's oxidative stress status (OSS) may serve a role in diagnosis and/or treatment (Pryor and Godber, 1991).

The major problems in determination of most of the free radicals of interest is that they are extremely reactive and possess very short life: if any produced in vivo, react at or close to their source of formation.Therefore, free radical activity is usually assessed by indirect methods such as measurement of the various end products resulting from interaction of free radicals with cellular components such as lipids, protein and DNA (Pryor and Godber, 1991; Slater, 1984).

Body has its own defense system against this continuous onslaught of free radicals in the form of some enzymes and cellular compounds known as endogenous antioxidants which gets exhausted in excessive generation of free radicals. The change in the levels of endogenous antioxidants is also related to the generation of free radicals. The estimations of the levels of superoxide dismutase, catalase and reduced glutathione provide a sound base for confirming the release of free radicals.

There are several methods for measuring OSS which includes the measurement of lipid per oxidation products such as malondialdehyde or TBARS in blood or urine (Gutteridge and

Tickner, 1978), vitamin E or vitamin C levels in blood fractions(including LDL) (Van Rensburg et al., 1989; Nierenberg, 1989), catalase or superoxide dismutase levels in blood fractions (Hageman et al., 1992; Larramendy et al., 1989), lipid peroxides in blood (Pryor, 1989), glutathione/glutathione disulfide in blood factions (Lang et al., 1989).

2.2 Cadmium Induced Oxidative Stress

Cadmium, a heavy toxic metal that is widely used in industry, affects human health through occupational and environmental exposure. It is present in soils, sediments, air and water and is listed by the US Environmental Protection Agency as one of 126 priority pollutants (1). It is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life of 10-30 years. It has been estimated that at least 512,000 U.S. employees each year work in an environment that potentially exposes them to cadmium. Cadmium is found more in food than air and water. The current federal minimal risk level (MRL) for cadmium – a level at which chronic exposure in humans is not likely to cause cancer or adverse health effects- is 0.2 μg/kg/day (14.0 μg the average adult). The average American diet in 1986 provided 0.4 μg/kg/day of cadmium. The overall range of dietary cadmium in Swedish diets in 1994-1996 was 2-175 μg/day and is estimated to be increasing at a rate of two percent per year. The World Health Organization has shown that dietary cadmium exposure has a very wide range: inhabitants of worldwide non polluted areas have a daily dietary intake of approximately 40-100 μg, while inhabitants of polluted areas may obtain 200 μg or more as an average daily intake.

The mechanism of cadmium-mediated toxicity has been the subject of investigations; although some uncertainties persist, sufficient evidence has emerged to provide a reasonable account of toxic processess. These involve two pathways, one for the initial injury produced by direct effects of cadmium and the other for the subsequent injuries produced by inflammation. Primary injury appears to be caused by the binding of cadmium to sulfhydryl groups which act as a critical molecule in mitochondria. Inactivation of thiol groups causes oxidative stress, mitochondrial permeability transition and mitochondrial dysfunction (Rikans and Yamano, 2000; Muller, 1986).

2.3 Antioxidants in Cadmium Toxicity

Metallothionein production is induced by the presence of metals, including cadmium, mercury, copper, gold, bismuth, and most powerfully, zinc (Coyle et al., 2002). Low level zinc treatments have been used in animal studies to induce metallothionein and protect against acute cadmium-induced hepatoxicity (Leber and Miya, 1976). Similarly, hepatocyte cell lines treated with zinc became resistant to cadmium-induced cell death as a result of metallothionein induction (Shimoda et al., 2001). In animals, both hepatic and intestinal metallothionein have been induced using oral zinc, and metallothionein induction using nontoxic zinc injections has been successful in reducing cadmium toxicity in animals (Onosaka et al.,2002). The induction of intestinal metallothionein in humans, using zinc acetate, is the mechanism for the FDA-approved treatment of Wilson's disease, an inherited condition where accumulation of copper in the liver, brain, and other organs leads to copper toxicosis (Brewer, 2000). The mechanisms of cadmium-induced renal damage result from the dissolution of the cadmium/metallothionein complex in the kidney, exposing renal tissue to unbound cadmium. Cadmium/cell membrane binding, cellular apoptosis of renal proximal tubules, increased calcium loss in the urine, and increased protein excretion are seen in animals given long-term doses of cadmium or repeated doses of cadmium/metallothionein complexes. Studies have also shown when the kidney is able to induce adequate de novo synthesis of metallothionein, no membrane damage occurs (Klaassen et al., 1999).

2.3.1 Zinc

Zinc has been used to induce renal metallothionein in animal studies and protects against cadmium/metallothionein-induced renal injury(Liu et al.,1996).Rats pretreated with zinc or copper have shown less sensitivity to cadmium toxicity, specifically in renal proximal tubule cells. Proteinuria caused by cadmium-metallothionein injections was more effectively reduced by pretreatment injections with zinc than with copper (Liu et al., 1994).Although there have been no human clinical trials with zinc or copper to assess metallothionein induction, zinc acetate, used to stimulate intestinal metallothionein in the treatment of Wilson's disease, is nontoxic in 150 mg daily doses and has minimal side effects(Brewer,

2000).In those without Wilson's disease, the possibility of inducing a copper deficiency with high doses of zinc is preventable with copper supplementation.

2.3.2 Alpha-Lipoic Acid

Alpha-lipoic acid (ALA), rejected in cadmium-exposed murine hepatocytes, was shown to protect cells from toxic effects of cadmium, including hepatocyte membrane damage, lipid peroxidation, and depletion of intracellular glutathione(Muller and Menzel,1990).These protective effects have also been seen in rats who had experimentally-depleted glutathione stores prior to cadmium exposure (Sumathi et al.,1996).Although the acute toxicity induced in these studies (150 μM or about 17 mg cadmium) is vastly different than low level chronic exposure in humans. Oxidant stress and glutathione depletion are also recognized toxic mechanisms of low level exposure(Bagchi et al.,1997;Shaikh et al.,1999).The authors of the first study concluded that dihydrolipoic acid (the reduced form of alpha-lipoic acid) is an effective extra- and intracellular chelator of cadmium in hepatocytes as a result of a significant decrease in intra- and extracellular levels of cadmium after ALA was added to the cells(Muller and Menzel,1990).The authors measured both intra- and extracellular cadmium/ lipoate and cadmium/dihydrolipoate complexes to conclude that cadmium was actually being removed from the hepatocytes by lipoic acid compounds themselves and not glutathione generated by lipoic acid. They also noted, however, that these effects occurred only at low levels of cadmium exposure and high levels of lipoic acid concentration(Muller and Menzel,1990).

2.3.3 Selenium

Plays a critical role in ridding the body of toxic substances and protecting the cells from their potentially harmful effects. It is the essential component of glutathione peroxidase, an enzyme present in most cells that chemically detoxifies hydrogen peroxide and other reactive oxygen intermediates produced in everyday metabolism. The antioxidant properties of this nutrient are associated with a reduced risk for cancer (particularly lung and intestinal cancers) and cardiovascular disease. Selenium decreases sensitivity to environmental toxins and is used as an adjunct therapy in the treatment of cancer. In addition, selenium has been

used therapeutically to treat allergies, viral infections, arthritis, heavy metal toxicity, cataracts, macular degeneration, and muscular dystrophy. Selenium works with other antioxidants such as vitamin C, beta-carotene, and vitamin E to rid the body of carcinogens.The theory that selenium and cadmium can form complexes has been substantiated by researchers in animal studies with concomitant selenium and cadmium exposure(Nehru and Bansal,1997; Sidhu et al., 1993).In a study with acute cadmium toxicity (8 mg/kg oral cadmium) and contaminant oral selenium supplementation (350 μg/kg sodium selenite), rats who received both had a 25-percent reduction in kidney cadmium. The ability of selenium to decrease the tissue burden of cadmium has been repeated in other animal studies(Nehru and Bansal,1997).Acute toxicity studies have found that, as a result of dosing selenium and cadmium at the same time, organ tissue levels of both metals increased and the toxic burden of cadmium decreased, possibly as a result of the inert nature of the cadmium/selenium complex(Environ Health Perspect,1978).A low-level cadmium exposure study in mice (1 ng/L drinking water) with a varied selenium diet revealed significant differences in cadmium retention(Andersen and Nielsen,1994).In mice that received a normal selenomethionine/sodium selenite diet (99.25 μg selenomethionine and 68 μg sodium selenite/kg food) the whole body retention of cadmium was less than half of the retention in mice on a low selenium diet (31.25 μg selenomethionine/kg food). For comparison, the average American gets approximately 65 μg of selenium per day through diet and supplementation.

Selenium supplementation has a known antioxidant action in cadmium toxicity. Selenium supplementation in acute cadmium toxicity has been shown to decrease lipid peroxidation in rat studies (Yiin et al., 2000) and has also been shown to increase the production of glutathione S-transferase and glutathione peroxidase in rhesus monkeys(Sidhu et al., 1993).The dosage of selenium in the primate study was far beyond what would be used in human trials, 500 μg/kg body weight, but the cadmium exposure was also elevated beyond possible environmental or occupational human exposure to 5 mg/kg body weight/day. Similar results-elevations of glutathione peroxidase and decreased whole body and renal burden of cadmium were found in rats given daily selenium supplementation of 350 μg/ kg body weight (Nehru and Bansal,1997). Selenium also appears to act in conjunction with

other antioxidants. When selenium was given to rats simultaneously with vitamin E and glutathione, the cadmium uptake in liver and kidneys was significantly inhibited(Rana and Verma,1996).Selenium has also been shown to decrease lipid peroxidation in testicular tissue of rats(Sugawara and Sugawara,1984),an effect relevant to human health due to the correlation of blood cadmium levels in men with decreases in sperm motility and alterations in sperm morphology(Telisman et al.,2000).

2.2 HYPERTENSION

The body's ability to maintain its blood pressure is vital to life. Chronic hypertension is a major coronary heart disease risk factor. Hypertension contributes to half a million strokes and over a million heart attacks each year. The higher the blood pressure above the normal 120/80 mmHg, the greater the risk of heart disease. This condition is sometimes referred to as the silent killer, since people usually cannot feel the physical effects of high blood pressure.

Other than a poor diet, obesity, certain diseases, sedentary lifestyle, and a genetic predisposition, there are several factors to hypertension.Lifesyle factors, environmental factors, and stress management can all play a role in hypertension.Lifestyle factors including smoking, alcohol, and coffee consumption, have been shown to increase blood pressure.

Environmental factors such as lead contaminated drinking water and cadmium toxicity have been shown to promote hypertension. Sources of cadmium include industrial commercial paints, cigarettes, foods and water polluted with this metal (Satarug et al.,2003; WHO/IPCS,1992; Waisberg et al.,2004).Cadmium accumulates particularly in kidneys and liver and has a biological half-life of 7-30 years (WHO/IPCS,1992; Sato and Kondoh,2002;Wittman and Hu,2002; Baker et al.,2003).Different organ impairments such as renal tubular dysfunction with impaired reabsorption of low molecular weight proteins caused by the retention of cadmium in the kidney have been established (Nordberg et al.,1992;Kaizu and Uriu,1995).Also cadmium-induced cardiac impairment has been reported(Kopp et al.,1980).Among the various chemical substances,Cd is one of the causes of hypertension (Glauser et al.,1976).

2.3 AIMS OF THE STUDY:

Recent evidences (U.S. environmental protection agency 2008; 11th report on carcinogens; Hayes and Andrew, 2007; Nogawa Koji et al., 2004; Upasani. 2001; Rikans and Yamano, 2000; Jarup et al., 1998) have shown that cadmium induces oxidative stress.

The mechanisms of cadmium toxicity are not completely understood, but some of the cellular effects are known. Cadmium is known to bind to the mitochondria of the cell and is capable of inhibiting both cellular respiration (by 75%) and oxidative phosphorylation (by 100%) at low concentrations. This mitochondrial toxicity can completely inhibit the hydroxylation of vitamin D in renal tissue at concentrations of 0.025 mmol. Fifty to sixty percent of exposed populations have been shown to have chromosomal damage.

Some of the specific changes that lead to tissue damage and death in chronic exposure have been related to oxidative stress and thiol depletion. Cellular damage results from cadmium binding to sulfhydryl groups in tissue, the production of lipid peroxides, and the depletion of glutathione. Cadmium also has a very high affinity for glutathione and can form a complex with glutathione that is eliminated in bile. Cadmium also inhibits the activity of antioxidant enzymes, including catalase, manganese-superoxide dismutase, and copper/zinc-superoxide dismutase. Cadmium-induced lipid peroxidation has been seen in animal studies in liver, kidney, brain, lung, heart and testis. Restoration of the distorted oxidant/antioxidant balance appears to provide a partial remedy to the toxic effects of cadmium caused mainly by enhanced oxidative stress. Several studies are underway to determine the effect of antioxidant supplementation following cadmium exposure.

An antioxidant is any substance that when present at low concentration compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The term oxidizable substrate includes almost everything found in living cells, including proteins, lipids, carbohydrates and DNA. The term reactive oxygen species is a collective one that includes not only oxygen centered radicals such as superoxide radical, singlet oxygen and hydroxyl radical, but also some potentially dangerous non-radicals like hydrogen peroxide. Fortunately, the human body makes several important antioxidants. The most important are ubiquinone and glutathione. Enzymes such as superoxide dismutase, catalase and glutathione

peroxidase also destroy free radicals. Antioxidants may act at different levels in the oxidative process by scavenging initiation of free radicals, binding metal ions, scavenging peroxyl radicals and removing oxidatively damaged bio-chemicals. Some antioxidants must be provided as micronutrients; they include ascorbic acid, beta-carotene, Coenzyme Q10, Vitamin E, Alpha Lipoic acid and trace metals such as selenium.

The challenge of this century is the rapid industrialization leading to increase in the level of pollutants like heavy metals in the environment. Heavy metals like cadmium are a part of smoke, pollution and industrial waste. These toxic metals lead to production of free radicals. Free radicals are energetically unstable and highly dangerous molecules. They lead to formation of dangerous peroxides which in turn cause genetic damage via DNA mutation, decline in immune function, increase in inflammatory conditions, growth and spread of cancers, oxidation of LD cholesterol leading to atherosclerosis, hormone disruption contributing to diabetes, hypertension and other systemic diseases. There is a need for supplementation of exogenous antioxidants to protect the body from the onslaught of free radical induced damage.

Antioxidants like coenzyme Q10, selenium and alpha lipoic acid are known to protect the body from damage caused by these harmful molecules.

Hence our aim was to study the effect of cadmium induced oxidative stress on endogenous enzymes in various organs and systems of the body and to investigate the beneficial effects of exogenous antioxidants like coenzyme Q10, selenium and alpha lipoic acid on cadmium induced toxicity.

Therefore the present work **"Effects of some antioxidants on cadmium induced cardiovascular and other toxic effects"** is aimed:

1- To study the effects of cadmium with special reference to the cardiovascular system.
2- To study the effect of alpha lipoic acid, selenium or coenzyme Q10 on cadmium induced cardiotoxicity and also to study the effect of these drugs on various antioxidant and hemodynamic parameters to justify whether the cardioprotective effect is due to the antioxidant mechanism of action.

3- To study the effects of cadmium alone on the levels of various enzymes and cellular constituents in organs like liver, lung, heart, kidney and brain and in serum of rats with reference to oxidative stress.

4- To find out the scope of exogenous antioxidants like alpha lipoic acid, selenium or coenzyme Q10 and their supplementation on the cadmium induced changes in levels of various enzymes and cellular constituents in tissues and serum with reference to oxidative stress.

5- To study the toxicological effects of these metals alone and in combination with coenzyme Q10, selenium or alpha lipoic acid on the basis of biochemical and pharmacological evidence.