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BIOCHEMICAL INFLUENCE OF OXIDATIVE STRESS OF CADMIUM ON TESTES IN RATS

Thesis Presented by

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M. Sc. Thesis

In

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ABSTRACT

Cadmium is ubiquitous environmental and occupational pollutant. It is mainly used in the manufacture of nickel–cadmium batteries, pigments and plastic stabilizers. This study was designed to investigate the ability of Cd to induce oxidative stress in the testes of male albino rats and the effects of Cd on male reproductive hormones and testicular histopathology. Ninety adult male albino rats were used in this study, they were divided into 3 equal groups, group 1: control; group 2: received Cd at 30 mg/L in drinking water for 8 weeks and group 3: received Cd at 60 mg/L in drinking water for 8 weeks. Rats were sacrificed at the end of the 4^{th}, 6^{th} and 8^{th} weeks for collection of blood and testicular samples.

The results revealed that Cd induced oxidative stress in the testes of male albino rats as evidenced by marked reduction in enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and non-enzymatic antioxidants such as reduced glutathione (GSH). Exposure to Cd also resulted in lipid peroxidation as indicated by significant increase in testicular MDA level. Testosterone, LH and FSH levels were also significantly decreased in blood as a result of exposure to Cd. Marked histopathological changes ranging from detachment and sloughing to vacuolization of the seminiferous tubules appeared in the testes of rats exposed to Cd.

Key words: Cadmium, oxidative stress, antioxidant enzymes, testosterone, rat, testes.

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INTRODUCTION

Environmental pollutants, including a variety of industrial and domestic chemicals, pesticides, fertilizers, heavy metals and ionizing radiation are major factors responsible for oxidative stress. Intoxication by heavy metals, particularly lead, cadmium, arsenic and mercury constitute serious threats to human health **(WHO, 1992 and Hu, 2000).**

Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, and fertilizer industries, and cigarette smoke (**Stohs and Bagchi, 1995).** With increasing production and utilization of Cd, not only industrial workers but also the general population is exposed to the toxic effects of Cd. This has been found to produce various pathological conditions like hepatic and renal dysfunction, testicular damage, respiratory and nervous system disorders **(Waisberg *et al.*, 2003 and Thompson and Bannigan, 2008).**

A variety of experiments have suggested that Cd causes oxidative damage to cells. Cd has been demonstrated to stimulate 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).**

Testicular changes due to Cd toxicity have been seen in a variety of animal models at different stages of growth and maturity. Cd-induced testicular pathogenicity includes severe hemorrhage, edema, necrosis and atrophy, as well as reduction in counts and motility of sperm and decreased testosterone concentrations in plasma and testes **(Koizumi and Li, 1992; Santos *et al.*, 2006 and Thompson and Bannigan, 2008).**

To protect the integrity of biological membranes from detrimental oxidative processes caused by free radicals, both enzymatic and non-enzymatic defense mechanisms are present in the cell **(Droge, 2002 and Halliwell and Gutteridge, 2007).** The components of these defense systems can be divided into two main groups: antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) **(Mates, 2000 and Halliwell and Gutteridge, 2007)** and small endogenous antioxidant molecules such as glutathione (GSH) **(Griffith, 1999).** Other exogenous antioxidants, such as tocopherols (Vitamin E) and ascorbate (Vitamin C), essential for the function of antioxidant enzymes, are of dietary origin **(Chen and Tappel, 1995; Tandon *et al.*, 2003 and Halliwell and Gutteridge, 2007).**

AIM OF WORK

The objective of this study was to investigate the toxic effect of cadmium upon testicular tissue through oxidative stress and lipid peroxidation mechanism. That oxidative stress would be investigated through;

1. Determination of changes in malondialdehyde (MDA), enzymatic antioxidants (GSH-Px and SOD) and non-enzymatic antioxidant (GSH).
2. Pollutant effect of cadmium on serum FSH, LH and testosterone hormones concentration would be investigated.
3. Also the study includes investigation of the histopathological harmful effect of cadmium upon testicular tissue.

REVIEW OF LITERATURE

1. Historical view:

Detailed historical information on cadmium (Cd) has recently been reviewed by **Nordberg (2009).** A German chemist Friedrich Strohmeyer discovered this metal in 1817. Forty years later, acute gastrointestinal and respiratory symptoms were observed in persons using cadmium carbonate powder as a polishing agent. Increased occupational exposure to Cd was observed after 1912, when the production of nickel-cadmium (Ni-Cd) batteries started in Sweden. Further investigations confirmed the toxic effects of Cd on the lungs, kidneys, and bones. Damage to the lungs in Cd-exposed workers was reported as early as the 1930s, while Nicaud observed osteomalacia and Friberg proteinuria and emphysema in the 1940s **(Friberg 1948 and Nordberg, 2009).**

However, it was Itai-itai (ouch-ouch) disease that demonstrated the dangerous dimensions of Cd as an environmental pollutant. It was an endemic bone disease characterized by fractures and severe pain related to exposure to Cd that occurred after World War II in Toyama Prefecture in Japan **(Tcuchiya, 1969 and Nordberg, 2009).** The clinical symptoms and signs of osteomalacia and osteoporosis, femoral pain, lumbago and skeleton deformations, renal tubular dysfunction, immune deficiencies, apathies, malabsorption, and anaemia were observed mostly in multiparous women living along the contaminated Jinzu River basin. Elevated levels of Cd were found in patients' urine. In 1968, the Japanese government acknowledged that the disease was as an environmental disease related to Cd-contaminated water released from a mine into the Jinzu River, which was used to irrigate rice fields. The disease was the result of several unfavourable factors:

consumption of Cd contaminated water and rice, low dietary intake of proteins, and essential element deficiencies due to multiple pregnancies. High Cd exposure levels were associated with adverse effects on the skeleton through toxic effects on kidney, and also, as was confirmed later, by direct Cd effects on bone tissue. For the first time, Cd pollution was shown to have severe consequences on human health. Subsequently, interest in Cd, initiated by Friberg's observations of renal damage in occupationally exposed workers, arrived on the scientific scene **(Friberg 1948).**

The discovery of a Cd-containing protein from horse kidney by **Margoshes and Vallee (1957)** marked the beginning of research of this low molecular weight, cysteine-rich protein named metallothionein by **Kagi and Vallee (1960).** Further studies confirmed the important role of metallothionein in the toxicokinetics and toxicodynamics of Cd and identified the kidney as one of the critical organs of Cd toxicity **(Sabolic *et al.*, 2010).**

2. Cadmium properties:

Cadmium is a naturally occurring element of relatively poor abundance (64^{th} amongst elements) in the earth's crust (0.1-0.5 ppm) with the symbol Cd and atomic number 48. While it occurs in air, water, soil as well as in tissues of plants and animals, it is not found in Free State. Cadmium is present primarily in ores of zinc, copper or lead, the extraction and processing of which releases large quantities of cadmium into the atmosphere, hydrosphere and soil thereby contaminating the human environment. It is toxic, nonessential and classified as a human carcinogen by the **North Carolina National Toxicology Program (2000).**

The physical and chemical properties of cadmium namely corrosion resistance (particularly in alkaline and seawater environments), low melting temperature, rapid ion electrical exchange activity, high electrical and thermal conductivity (in both alloy and oxide forms) make it suitable for incorporation into batteries, alloys and for electroplating, welding, electrical, and nuclear fission applications **(Martelli *et al.*, 2006).**

Cadmium is classified as a toxic element without any beneficial role in human physiology. Several studies have shown the role of Cd as an essential metal in ruminants **(Memisi *et al.*, 2008).** It was recently reported that some marine algae contain a form of the enzyme carboanhydrase with cadmium instead of zinc (Zn) in their active sites **(Lane *et al.*, 2005).**

Cadmium has also found its place in the rapidly expanding field of nanotechnology, which is therefore likely to become yet another source of its toxicity. Cadmium-containing nanoparticles, known as CdSe or CdTe-core quantum dots, have numerous biomedical applications, especially in the diagnosis of cancer, due to their unique optical and electrical properties. Despite their potential to revolutionise medical therapy, Cd-containing quantum dots are potentially toxic and their use presents substantial risk **(Derfus *et al.*, 2004; Kirchner *et al.*, 2005; Green and Howman, 2005 and Smith *et al.*, 2008).**

3. Occupational and environmental exposure to cadmium:

Cadmium (Cd) is an earth's crust natural element and is usually found as a mineral in combination with other elements such as oxygen, chlorine, or sulfur. Over the past two centuries, anthropogenic and industrial activities have led to high emissions of Cd into the environment at concentrations significantly exceeding those originating from natural sources