



Cairo University
Faculty of Veterinary Medicine
Department of Toxicology, Forensic
Medicine and Veterinary Regulations



Effect of Calcium Disodium EDTA Nanoparticles against Cadmium Toxicity in Rats

Thesis presented by

Safa Mohamed Saleh Ibrahim

M.V.Sc. (2014)

For Ph.D. V.Sc. degree

(Toxicology, Forensic Medicine and Veterinary Regulations)

Under supervision of

Prof. Dr. Eiman M. El-Saied

Professor and Chairman of Toxicology, Forensic Medicine and Veterinary
Regulations Department, Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Osama S. El-Tawil

Professor of Toxicology and Forensic
Medicine, Faculty of Veterinary
Medicine, Cairo University

Prof. Dr. Manal B. Mahmoud

Professor and Chairman of the
Immune Section, Research Institute
for Animal Reproduction

2018



Cairo University
Faculty of Veterinary Medicine
Department of Toxicology,
Forensic Medicine and Veterinary Regulations

APPROVAL SHEET

This is to approve that the dissertation presented by "**Safa Mohamed Saleh Ibrahim**" in Cairo University entitled "**Effect of Calcium Disodium EDTA Nanoparticles against Cadmium Toxicity in Rats**" for the degree of **Ph.D.V.Sc.** (Toxicology, Forensic Medicine and Veterinary Regulations) has been approved by the examining committee.

Examining Committee

Signature

Prof. Dr. Ali Hidar Abo-Hadid

Professor of Forensic Medicine and Toxicology,
Faculty of Veterinary Medicine, Zagazig University

Prof. Dr. Abd Al-Azim Ali Ahmed Khalaf

Professor of Toxicology, Forensic Medicine and
Veterinary Regulations, Faculty of Veterinary
Medicine, Cairo University

Prof. Dr. Eiman Moustafa El-Saied

Professor and Chairman of Toxicology, Forensic
Medicine and Veterinary Regulations Department,
Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Osama Samir El-Tawil

Professor of Toxicology, Forensic Medicine and
Veterinary Regulations, Faculty of Veterinary
Medicine, Cairo University



Cairo University
Faculty of Veterinary Medicine
Department of Toxicology, Forensic
Medicine and Veterinary Regulations



SUPERVISION SHEET

SUPERVISORS

Prof. Dr. Eiman Moustafa El-Saied

Professor and Chairman of Toxicology, Forensic
Medicine and Veterinary Regulations Department,
Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Osama Samir El-Tawil

Professor of Toxicology, Forensic Medicine and
Veterinary Regulations, Faculty of Veterinary
Medicine, Cairo University

Prof. Dr. Manal Bahaa El-Din Mahmoud

Professor and Chairman of the Immune Section,
Research Institute for Animal Reproduction



Cairo University
Faculty of Veterinary Medicine
Department of Toxicology, Forensic
Medicine and Veterinary Regulations

Name : Safa Mohamed Saleh Ibrahim
Date of birth: 20-09-1985
Nationality : Egyptian
Degree : Doctor of Philosophy of veterinary science Degree
Specification: Toxicology, Forensic Medicine and Veterinary Regulations
Title: Effect of Calcium Disodium EDTA Nanoparticles against Cadmium Toxicity in Rats
Supervision: Prof. Dr. Eiman Moustafa El-Saied
Prof. Dr. Osama Samir El-Tawil
Prof. Dr. Manal Bahaa El-Din Mahmoud

ABSTRACT

In this study, the median lethal dose (LD_{50}) of cadmium sulfate was determined firstly. Then, the animals were classified at the start of the experiment, into two groups; a control (contains 15 rats) and an intoxicated one (contains 45 rats), which received drinking water contains 30 ppm cadmium for 10 weeks. At the end of the 6th week of the experiment, the intoxicated group was subdivided equally into three groups. The second and third groups respectively were injected intraperitoneally with 50 mg/kg/day macroparticles or nanoparticles $CaNa_2EDTA$ for 4 courses (4 days each) with an interval of 3 days between the courses. Results showed that, the estimated median lethal dose (LD_{50}) of cadmium sulfate for adult female rats was 240 mg/kg. Cadmium significantly reduced body weight gain and food consumption and induced a marked depression. Cadmium also induced histopathological changes in liver, kidney and bones and decreased bone mineralization as revealed by X-ray examination. Serum urea and creatinine concentrations were significantly increased by cadmium. Concentrations of cadmium and metallothionein in serum and urine were significantly increased in cadmium-intoxicated rats. Cellular immune response (as determined by the total and differential leucocytic counts, the phagocytic activity of neutrophils, lymphocytes transformation and serum lysozyme activity) were significantly affected. Humoral immune response as estimated by the measurement of electrophoresis pattern of serum proteins and cytokines production (serum IL-2 and IL-6) was significantly decreased by cadmium. These cadmium-associated alterations were markedly alleviated by $CaNa_2EDTA$ nanoparticles treatment, while $CaNa_2EDTA$ macroparticles treatment induced a mild protective effect when compared with the nanoparticles form. These findings suggested that $CaNa_2EDTA$ nanoparticles could be used as an effective chelating agent for cadmium because they have a more powerful chelating capacity and thus could modulate the development of severe toxic effects of cadmium in rats. In addition, these nanoparticles reduce the substantial risks associated with $CaNa_2EDTA$ macroparticles therapy.

Keywords: Cadmium, $CaNa_2EDTA$, Nanoparticles, Female rats

ACKNOWLEDGMENT

*I would like to give special thanks to **Prof. Dr. Eiman Moustafa El-Saied**, Prof. and Chairman of Toxicology, Forensic Medicine and Veterinary Regulations Department, Faculty of Veterinary Medicine, Cairo University for suggesting the research point and for gentle personality that gave me great strength throughout my work. I would like to thank her for her time, experience and kind advices. I would like to give her all best wishes.*

*I would like to give special thanks to **Prof. Dr. Osama Samir El-Tawil**, Prof. of Toxicology, Forensic Medicine and Veterinary Regulations, Faculty of Veterinary Medicine, Cairo University for supplying me with his valuable scientific experience and for helping me to solve problems facing me during the experiments and for continuous emotional encouragement and help.*

*I would like to give special thanks to **Prof. Dr. Manal Bahaa Eldin Mahmoud**, Professor and Chairman of the Immune Section, Research Institute for Animal Reproduction for supplying me with scientific experience needed for the experiments. I would like to give special thanks to her for her time and help.*

*I would like to thank **Prof. Dr. Sahar Samir Abd El-Rahman**, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University for performing the light microscopic examination and for her emotional encouragement.*

*I would like to thank all members of the Immune Section, Research Institute for Animal Reproduction specially **Dr. Abeer Anwer, Dr Lila and Dr. Enas Gamal Eldin** for hard working with me and for continuous emotional encouragement and help.*

I would like to thank all members of Toxicology, Forensic Medicine and Veterinary Regulations Department, Faculty of Veterinary Medicine, Cairo University for their continuous emotional encouragement and help.

Finally, I am very thankful to my family for supporting me with their love, time, kind advice and emotional encouragement.

Safa Mohamed Saleh

CONTENTS

	Page
INTRODUCTION AND AIM THE WORK OF	1
LITERATURE REVIEW	4
MATERIALS AND METHODS	23
RESULTS	46
DISCUSSION	106
REFERENCES	117
ENGLISH SUMMARY	136
ARABIC SUMMARY	139

INTRODUCTION AND AIM OF THE WORK

Cadmium 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. Cadmium is a transitional metal that exists in different oxidational or transitional states. It occurs in rock erosion and abrasion and volcanic eruptions, fossil fuels and particularly non-ferrous mining and metal industries. Cadmium stimulates and binds to various biological components such as proteins and nonprotein sulfhydryl groups, macromolecules and metallothionein. Cadmium-induced peroxidation caused the release of free oxygen radicals, which cause the stimulation and destruction of sensitive macromolecules and tissues. Cadmium toxicity is associated with several clinical complications, renal dysfunction, bone diseases and hepatic dysfunction. Renal tubular damage is probably the most common adverse effect. The liver injury is also of acute toxicity dominated by apoptosis and necrosis. Cadmium can also cause various forms of diseases; osteomalacia and osteoporosis, hypertension, arteriosclerosis, anemia and Cancer (**Llobet *et al.*, 1998; Klassen *et al.*, 1999; Jarup *et al.*, 2000 and Lafuente *et al.*, 2000**).

The immune system suffers from Cd-induced impairment at several levels. Prenatal Cd exposure may impair postnatal T cell production and response to immunization, as well as dysregulated thymocyte development. Post-natal Cd exposures induce cell cycle arrest and apoptosis in splenocytes. Cd induces increased rates of autoimmunity, increased production of nonspecific antibodies, and decreased production of antigen-specific antibodies. Lymphocyte proliferation and natural killer cell activity are also suppressed by Cd (**Fortier *et al.*, 2008; Chatterjee *et al.*, 2009; Ohsawa, 2009 and Hanson *et al.*, 2012**).

Several chelators are used to treat Cd toxicity as Ethylene-diamine-tetraacetic acid (EDTA), Dimercaptosuccinic acid (DMSA), 2,3-Dimercapto-1-propanesulfonic acid (DMPS) and British Anti-Lewisite (BAL). BAL is more toxic than its derivatives, DMPS and DMSA, and is seldom used clinically. *In vitro* and *in vivo* studies suggest that EDTA is superior to DMSA in mobilizing intracellular Cd. The risks associated with CaNa_2EDTA therapy are substantial, including renal failures, arrhythmias, tetany, hypocalcaemia, hypotension, bone marrow depression, prolonged bleeding time, convulsions and respiratory arrest. Other adverse effects may include fatigue, headache, fever, nasal congestion, lacrimation, mucocutaneous lesions, glycosuria, myalgia, hepatotoxicity, increased urinary frequency, abnormal changes in Electrocardiography and gastrointestinal symptoms. Prolonged treatment with CaNa_2EDTA results in depletion of essential metal as Zn, Cu and Mn **(Flora and Tandon, 1990; Ibim *et al.*, 1992; Knudtson *et al.*, 2002 and Lin-Tan *et al.*, 2007).**

For a few years now, nanotechnology has emerged as an area of science and technology that is leading us to a new industrial revolution. Nanotechnology is defined as scientific and technological development at the atomic and molecular levels, in the range of about 1-100 nm, to obtain a fundamental understanding of phenomena and materials on a nanoscale and to create and use structures, devices and systems that have novel properties and functions due to their size. The aim of nanotherapy is to drive nanosystems containing recognition elements to act or transport and release drugs exclusively in cells or affected areas in order to achieve a more effective treatment and minimizing side effects. Nanoparticulate drug delivery systems are being used to alter the drug's biopharmaceutics and pharmacokinetics such as drug absorption, distribution, metabolism, and elimination **(Schek *et al.*, 2004 and Jain, 2005).**

Therefore, the present study aims to investigate the effect of CaNa_2EDTA nanoparticles against cadmium toxicity in female rats aiming to minimize its side effects and optimize its use as a chelating agent for cadmium toxicity. The predictive protective effect was compared with CaNa_2EDTA macroparticles.

LITERATURE REVIEW

Cadmium (Cd) is a naturally occurring metal situated in the Periodic Table of the Elements between zinc (Zn) and mercury (Hg), with chemical behavior similar to Zn. Cadmium is classified as a transition metal; it generally exists as a divalent cation, complexed with other elements. Cadmium metal (Cd^{2+}) refined from the ore is a silver-white, blue-tinged lustrous heavy metal solid at room temperature. Cadmium metal and its oxides are insoluble in water. Some cadmium salts are water soluble such as cadmium chloride, cadmium sulfate and cadmium nitrate; other insoluble salts can become more soluble by interaction with acids, light or oxygen. Solid cadmium is inflammable but powdered cadmium will burn and release corrosive and toxic fumes **(NTP, 2004 and HSDB, 2006)**.

Cadmium is listed as number seven of 275 of the most hazardous substances in the environment, behind arsenic, lead, mercury, vinyl chloride, polychlorinated biphenyls and benzene by the Agency for Toxic Substances and Diseases Registry **(ATSDR, 2007)**. Cadmium (Cd) is classified by International Agency for Research on Cancer of USA (IARC) as a human carcinogen. It is considered to be one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life **(IARC, 1993 and Patrick, 2003)**.

Cd exists in the earth's crust, usually being found as an impurity in Zn or lead (Pb) deposits, and therefore being produced primarily as a byproduct of their smelting. Commercially, Cd is used in television screens, lasers, batteries, paint pigments, cosmetics, and in galvanizing steel, as a barrier in nuclear fission, and was used with zinc to weld seals in lead water pipes prior to the 1960s **(Hans, 1995 and U. S. Geological Survey, 2012)**.

Cadmium does not degrade in the environment and can enter the food chain, which steadily increases the risk of exposure. Human activities such as mining, waste incineration, fossil fuel combustion, and application of phosphate fertilizers and sewage sludge that contain Cd significantly contribute to the contamination of soils and consequently is taken up by cultivated plants. When cadmium is released into the atmosphere by smelting or mining or some other processes, it can be associated with respirable-sized airborne particles and can be carried long distances. Wheat and rice, green leafy vegetables, and potatoes contain higher concentration than other foods from plants and account for more than 80 % of Cd intake by food. Cadmium is also present in high concentrations in fish and sea food (molluscs and crustaceans) and also found in meat, especially sweetmeats such as liver and kidney. Cadmium concentrations in drinking water supplies are typically less than 1 µg/l or 1 part per billion (ppb). Groundwater seldom contains high levels of cadmium unless it is contaminated by mining or industrial wastewater, or seepage from hazardous waste sites. Soft or acidic water tends to dissolve cadmium and lead from water lines; cadmium levels are increased in water stagnating in household pipes (**ATSDR, 1999 and Nawrot *et al.*, 2010**).

Toxicokinetic of Cadmium

The principal factor determining how much cadmium is absorbed is the route of exposure. Once exposed, how much cadmium is absorbed depends on many factors such as age, gender, smoking, and nutritional status. As a cumulative toxin, cadmium body burden increases with age. Women have been shown to have higher blood levels of cadmium than men. Typically women, with lower iron status, are believed to be at risk for greater absorption of cadmium after oral exposure (**Olsson *et al.*, 2002**).

In the lungs, 10% to 50% of an inhaled dose is absorbed, depending on particle size, solubility of the specific cadmium compound inhaled, and duration of exposure. Absorption is least for large (greater than 10 μm and water-insoluble particles, and greatest for particles that are small (less than 0.1 μm) and water soluble. A high proportion of cadmium in cigarette smoke is absorbed because the cadmium particles found in that type of smoke are very small. Most orally ingested cadmium passes through the gastrointestinal tract unchanged as normal individuals absorb only about 6% of ingested cadmium, but up to 9% may be absorbed in those with iron deficiency. Also, cadmium in water is more easily absorbed than cadmium in food (5% in water versus 2.5% in food). The presence of elevated zinc or chromium in the diet decreases cadmium uptake. Absorption through the skin is not a significant route of cadmium entry; only about 0.5% of cadmium is absorbed by the skin **(ATSDR, 1999; Järup, 2002 and US EPA, 2006)**.

Absorbed Cd circulates in red blood cells or bound to albumin in plasma. About 30% deposits in the liver and 30% in the kidneys, with the rest distributed throughout the body. In the liver it may induce and bind metallothionein (MT), this complex is released slowly into circulation and then accumulates in kidneys. It may also be stored in bone, pancreas, and adrenals and in the placenta; however, liver and kidney together contain about 50% of the body's accumulation of cadmium. There is little or no metabolism of cadmium, although it binds to various macromolecules and proteins **(RAIS, 1991 and HSDB, 2006)**.

The clearance half-life of cadmium is twenty-five years. The half life of cadmium in the blood has been estimated at 75 to 128 days, but this half life primarily represents deposition in organs, not clearance from the body. The biologic half-life of cadmium in the kidney is estimated to be between 6 to 38 years; the half life of cadmium in the liver is between 4 and 19 years. The total

cadmium body burden at birth is non-detectable; it gradually increases with age (**Järup *et al.*, 1983 and CDC, 2005**).

Absorbed cadmium is eliminated from the body primarily in urine. The rate of excretion is low, probably because cadmium remains tightly bound to metallothionein which, is almost completely reabsorbed in the renal tubules. Small amounts of cadmium usually conjugated with glutathione, cysteine or metallothionein are excreted in the feces. The daily excretion of cadmium from the body (mainly by the kidneys) does not exceed 0.01% of the amount of this element consumed in the diet. Because excretion is slow, cadmium accumulation in the body can be significant. Cadmium concentration in blood reflects recent exposure; urinary cadmium concentration more closely reflects total body burden. However, when renal damage from cadmium exposure occurs, the excretion rate increases sharply, and urinary cadmium levels no longer reflect body burden (**Järup, 2002; Zalups and Ahmad, 2003 and USEPA, 2007**).

With increasing evidence of cadmium's toxicity, international agencies have sought to regulate its exposure. Occupational Safety and Health Administration (OSHA) has established workplace levels to protect the health of people occupationally exposed to cadmium. The OSHA limits are Permissible Exposure Limit- Time-Weighted Average (PEL- TWA): 5µg/ m³ (fumes) and The National Institute of Occupational Safety and Health (NIOSH) has set an Immediately Dangerous to Life and Health level, which is 9mg/m³ (**Satoh *et al.*, 2002; NTP, 2004 and NIOSH, 2006**).

Many health agencies have set exposure standards designed to protect the general public from excess cadmium exposure from various sources. Maximum limit of cadmium in bottled water is 0.005 mg/L. Chronic durational oral minimal risk level (MRL) is 0.1 µg/kg/day of cadmium based on its renal effects. This MRL

standard states how much cadmium can be taken in orally chronically without risk of adverse health effects. Food – Reference dose is 1×10^3 mg/kg/day and Water - Reference dose for human exposure is 5×10^4 mg/kg/day. Reference dose is an estimate of a daily exposure to the general population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. Drinking water - maximum contaminant level for cadmium is 0.005 mg/L. The ceiling for the amount of cadmium that can be applied to land is 85 mg/kg fill material. Tolerable weekly intake for cadmium is 7 µg/kg/week (ATSDR, 1999; NTP, 2004 and US EPA, 2006).

Toxicodynamic of Cadmium

Cadmium is known to increase oxidative stress by being a catalyst in the formation of reactive oxygen species, increasing lipid peroxidation, and depleting glutathione and protein-bound sulfhydryl groups. Cadmium also can stimulate the production of inflammatory cytokines and down regulates the protective function of nitric oxide formation (Navas-Acien *et al.*, 2004).

Toxicity could result from Cadmium interacting with cellular components even without entering the cell, but by interaction with receptors on their surface. Cadmium forms covalent and ionic bonds with atoms of sulfur, oxygen and hydrogen present in the sulfhydryl groups, disulfide, carboxyl, imidazole or multiple amino compounds present in the cells, causing significant disruption of their homeostasis (Beyersmann and Hechtenberg, 1997 and Bertin and Averback, 2006).

The main target organelle of cadmium is the mitochondria. Cadmium enters the mitochondria through calcium channels and induces conformational changes in proteins located in the membrane by binding to thiol groups. Consequently interfere with oxidative phosphorylation and alter its membrane permeability

leading to reduction in membrane potential, decrease in cellular adenosine Triphosphate (ATP) levels, disturbances in homeostasis of calcium, sodium, potassium and ultimately leakage of cytochromes, Iron (II) ions leading to increased reactive oxygen species (ROS) and variety of effects. Consequences include increased production of reactive oxygen species and changes in the expression of different genes, which trigger cell cycle arrest, differentiation, immortalization or apoptosis **(Koizumi, 1996; Li *et al.*, 2000 and Chan *et al.*, 2006)**.

In all likelihood, cadmium being a divalent cation is accumulated by transport mechanisms developed for essential metals. From physical and chemical properties, those metals are most likely to be zinc, iron, magnesium, manganese, calcium and selenium. Cadmium may interact with these elements and cause their secondary deficit thereby disrupting metabolism, resulting in the final morphological and functional changes in many organs **(Brzóska and Moniuszko-Jakoniuk, 1998 and 2001)**.

Cadmium causes mutations, deoxyribonucleic acid (DNA) strand breaks, chromosomal damage, cell transformation and impaired DNA repair in cultured mammalian cells. Cadmium is known to modulate gene expression and signal transduction. It is known that metals and metalloids cause cancer primarily by direct interaction with DNA. Since cadmium is poorly mutagenic it may well act as an epigenetic or indirect genotoxic carcinogen. Yet another impact of cadmium on the activation and gene expression may be inhibition of DNA methylation. In the case of hypomethylation, over expression and excessive synthesis of protein products occur which are responsible for increased cell proliferation, which may result in the development of malignancies **(Waalkes and Misra, 1996; Takiguchi *et al.*, 2003 and Waisberg *et al.*, 2003)**.