PROTECTIVE EFFECT OF CALCIUM DISODIUM EDTA NANOPARTICLES AGAINST NEPHROTOXICITY OF CADMIUM IN FEMALE RATS

By

Safa M. Saleh[1], Eiman M. El-Saied[1], Osama Samir El-Tawil[1] and Manal B.Mahmoud[2]

[1]Toxicology, Forensic Medicine and Veterinary Regulations Department, Faculty of Veterinary Medicine, Cairo University, Egypt

[2]Immune Section, Research Institute for Animal Reproduction, Egypt

ABSTRACT

In this study, the toxic effects of cadmium sulfate on adult female Sprague Dawley rats as well as the ameliorating effects of $CaNa_2EDTA$ nanoparticles against such toxicity were investigated. The predictive protective effect was compared with $CaNa_2EDTA$ macroparticles. Animals were classified at the start of the experiment, into two groups; a control (contains15 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. The median lethal dose (LD_{50}) of cadmium sulfate was determined. The effect of $CaNa_2EDTA$ nanoparticles against cadmium toxicity was investigated by the assessment of clinical signs and symptoms, body weight gain, the levels of serum urea and creatinine, cadmium concentrations in serum and urine and histopathological examination of liver, kidney and femur. The obtained results revealed that the estimated LD_{50} for cadmium sulfate in adult female Sprague Dawley rats was 240mg/kg. Cadmium significantly reduced body weight and induced a marked depression in the intoxicated rats. The levels of serum urea and creatinine were significantly increased. Serum and urine cadmium concentration were significantly increased in cadmium-intoxicated rats. Cadmium also induced histopathological changes in the liver, kidney and bones examination. These alterations were markedly alleviated by $CaNa_2EDTA$ treatment, while $CaNa_2EDTA$ nanoparticles treatment induced a marked protective effect when compared to the macrooparticles form. In conclusion, 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 than macroparticles and thus could modulate the development of severe toxic effects of cadmium in rats.

Keywords:

Cadmium, CaNa$_2$EDTA, Nanoparticles, Female rats.

INTRODUCTION

Cadmium (Cd) is one of the most important environmental and occupational cumulative metallic toxicants of long elimination half-life of 10-30 years and is widely dispersed in the environment. It occurs in rock erosion and abrasion and volcanic eruptions, fossil fuels and particularly non-ferrous mining and metal industries **(Friberg *et al.*, 1986; Donald *et al.*, 1996 and Järup *et al.*, 2000).** High-level exposure to this toxic heavy metal is usually the result of environmental contamination from human activities. The compound has varying degrees of solubility, absorption and toxicity **(Falk *et al.*, 1990).** Cadmium stimulates and binds to various biological components such as proteins, non-protein sulfhydryl groups, macromolecules and metallothionein **(Klassen *et al.*, 1999).** Cadmium-induced peroxidation results in the release of free oxygen radicals **(Llobet *et al.*, 1998)** which stimulate and destruct sensitive macromolecules and indeed tissues **(Lafuente *et al.*, 2000).** Cadmium toxicity is dependent on dose, duration and route of exposure. It is associated with renal, hepatic, neurological, skeletal and other toxic effects, including reproductive toxicity, heart diseases, genotoxicity and carcinogenicity **(Waalkes *et al.*, 1999 and Matović *et al.*, 2011).** Cadmium has also inhibits the activities of various pancreatic proteases **(Shimada *et al.*, 2000).**Several chelators are used to treat cadmium toxicity. Clinically available chelators include Ethylenediaminetetraacetic acid (EDTA), 2,3-Dimercapto-1-propanesulfonic acid (DMPS), Dimercaptosuccinic acid (DMSA), and British Anti-Lewisite (BAL). BAL is more toxic than its derivatives, DMPS and DMSA, so, it is seldom used clinically. *In vitro* **(Borenfreund and Puerner, 1986)** and *in vivo* **(Andersen *et al.*, 1988)** studies suggest that EDTA is superior to DMSA in mobilizing intracellular cadmium. The risks associated with Calcium Disodium EDTA (CaNa$_2$EDTA) therapy are substantial, including renal failures, arrhythmias, tetany, hypocalcaemia, hypotension, bone marrow depression, prolonged bleeding time, convulsions and respiratory arrest. **(Knudtson *et al.*, 2002**). Prolonged treatment with CaNa$_2$EDTA results in depletion of essential metal, especially Zinc, Copper and Manganese. However, there are some reports highlights the efficacy of EDTA in chronic renal artery diseases **(Ibim *et al.*,**

1992). Nanotechnology has emerged as an area of science and technology that is leading us to a new industrial revolution. 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 **(Fortina *et al.*, 2005 and Jain, 2005).** Therefore, the present study aims to investigate the protective effect of CaNa₂EDTA nanoparticles against cadmium toxicity on adult female rats in order to minimize the side effects of CaNa₂EDTA therapy and optimize its use as a chelating agent for cadmium toxicity. The predictive protective effect was compared with CaNa₂EDTA macroparticles.

MATERIAL AND METHODS

Animals:

Thirty adult female Sprague Dawley rats weighing (200 ± 15 g) were used for determination of the oral Median Lethal Dose (LD$_{50}$) of cadmium sulfate. Another sixty female rats of the same strain weighing (150 ± 15 g) were used for the toxicological assessment. The animals were kept under observation for a week before the start of the experiment at suitable laboratory conditions in the normal day light and on diet and tap water *ad libitum*. Animals received humane care in compliance with the guidelines for the care and use of laboratory animals as approved by the International Animal Care and Use Committee, Cairo University (CU- IACUC) with approval number (CU- II- 20-16).

All chemicals and reagents used in this study were analytically pure and were purchased from Sigma-Aldrich Co. (St. Louis, USA), Aldrich (Germany) and El-Nasr Co. (Cairo).

Cadmium sulfate:

It was added to the drinking water at a concentration of 60 ppm (mg/L) which provides 30ppm cadmium. The selected concentration was nearly equivalent to $^1/_{20}$ of the estimated LD$_{50}$ which is recommended for sub-chronic toxicity **(Samy *et al.*, 2014)** and at the same time, this concentration was less than some contaminated areas in Egypt **(El-Shehawi *et al.*, 2007).**

Calcium disodium EDTA:

Calcium disodium EDTA macroparticles and nanoparticles were intraperitoneally injected at a dose of 50 mg/kg/day for 4 courses (4 days each) with an interval of 3 days between the courses **(Foreman, 1961).** Calcium disodium EDTA nanoparticles were purchased from Nano-Tech, Dreamland, 6 October, Giza, Egypt. CaNa₂EDTA was prepared in form of nanospheres of mean size 25±5 nm. They were characterized by transmission electron

microscopy (TEM) as showed in Fig. (1). Nano CaNa$_2$EDTA was prepared by nanoprecipitation method. CaNa$_2$EDTA was dissolved in polyvinyl alcohol solution at a definite concentration, and the solution was poured into water-immiscible non-solvent (chloroform) under continuous stirring until a cloudy suspension was formed. Precipitation was formed immediately upon mixing. Then the solution was dried and re-suspended in water **(Kumar *et al.,* 2011).**

Experimental Design:

Firstly, rats were classified into two groups; a control (contains15 rats) and an intoxicated one (contains 45 rats), which received drinking water contains 60 ppm cadmium sulfate (which provides 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$_2$EDTA for four courses (4 days each) with an interval of 3 days between the courses.

All groups were observed daily during the whole period of the experiment for apparent clinical signs of toxicity. Every two weeks, ten animals from each group were randomly selected, weighed and then blood samples for serum separation were collected from the inner census of the eye; in addition, 24-hour urine samples were collected. At the end of the experiment, all animals were anesthetized by intra-peritoneal injection of 100 mg/kg ketamine hydrochloride **(Fischer, 2008)** followed by decapitation and then were sacrificed. Specimens from liver, kidney and femur were randomly collected from sacrificed rats and fixed in 15% buffered formalin for histopathological examination. Paraffin-embedded sections were prepared and stained according to **Kieman, (1999).**

Determination of the Median Lethal Dose:

The median lethal dose (LD$_{50}$) value was calculated according to the arithmetical method stated by **Turner, (1965).**

Biochemical analysis:

Serum urea and creatinine concentrations were determined by using readymade kits obtained from EGY-CHEM Co., Badr City, Egypt according to the method described by **Vassault *et al.,* (1986) and Young *et al.,* (1975)** respectively. Cadmium concentration in serum and urine was measured by UNICAM 969 Atomic Absorption Spectrophotometer. The samples

were prepared according to the method recommended by **Graig and Wayne, (1984).**

Statistical analysis: The obtained results were presented as means ± SE. One-way analysis of variance (ANOVA) test was used for comparisons between different groups followed by LSD comparisons test. The level of significance was set at $P \leq 0.05$ using SPSS software (version 16.0).

RESULTS

Median Lethal Dose:

The calculated value of LD_{50} of cadmium sulfate for adult female Sprague Dawley rats was illustrated in the following table and equation.

Group	Dose (mg/Kg)	Number of dead animals	Dose difference (a)	Mean mortality (b)	Probit (a×b)
1	Vehicle	0			
2	300	1	100	0.5	50
3	400	1	100	1	100
4	500	0	100	0.5	50
5	600	0	100	0	0
6	700	2	100	1	100

$$LD_{50} = \text{Least lethal dose} - \sum \frac{(a \times b)}{N}$$

$$LD_{50} \text{ value of cadmium sulfate} = 300 - \frac{300}{5} = 240 \text{ mg/kg}$$

Clinical Signs and Body Weight

Rats of the control group were active and alert; in contrast, cadmium treated rats showed marked depression. After treatment with $CaNa_2EDTA$ macroparticles, the rats still exhibited depression, while with nanoparticles form; the rats were active and alert nearly as in those of the control group. Frequent urination was observed in the $CaNa_2EDTA$ macroparticles and nanoparticles treated rats. No mortality was recorded in the control and the other experimental groups. Cadmium intoxicated rats showed a significant decrease in their body weight when compared to the control rats. Treatment with $CaNa_2EDTA$ nanoparticles exerted a significant increase in body weight when compared to the cadmium group. In contrast, the body weights of the $CaNa_2EDTA$ macroparticles treated rats were near those of the cadmium intoxicated rats with insignificant difference between them (Table 1).

j. Egypt. net. med. Assoc 78, no 3, 443 – 462 (2018)

447

Table (1): Mean values ± S.E of the body weight (g) in control and experimental rats received drinking water contain 30 ppm cadmium and intraperitoneally injected with 50mg/kg/day CaNa$_2$EDTA macroparticles or nanoparticles at the end of the 6th till the 10th week of the experiment for 4 courses (4 days each) with 3 days interval between the courses

Groups / Time	Control	Cadmium	Cadmium and CaNa$_2$EDTA Macroparticles	Cadmium and CaNa$_2$EDTA Nanoparticles
2nd week	146.1 ± 4.5 a	135.5 ± 4.2 a		
4th week	161.4 ± 4.2 a	145.4 ± 4.7 b		
6th week*	178.0 ± 5.4 a	155.7 ± 3.8 b		
8th week	189.1 ± 4.1 a	165.5 ± 4.1 b	170.5 ± 3.8 b	181.6 ± 3.4 a
10th week	192.8 ± 6.9 a	172.6 ± 4.0 b	180.5 ± 5.4 abc	190.3 ± 4.1 ac

Values have different scripts at the same row are significantly different at P ≤ 0.05 (n=14)

*Start of the treatment with CaNa$_2$EDTA macroparticles or nanoparticles.

Serum Urea Concentration:

Serum urea concentration of the cadmium group showed a significant increase than that of the control group. Rats treated with CaNa$_2$EDTA nanoparticles showed a marked decrease in their serum urea concentration when compared to the cadmium-intoxicated rats especially at the 10th week of the experiment in which insignificant increase from that of the control rats was recorded. CaNa$_2$EDTA macroparticles treatment reduced the elevation in the urea concentration exerted by cadmium, but to a lesser degree than the nanoparticles form (Table 2).

Table (2): Mean values ± S.E of the serum urea concentration (mg/dl) in control and experimental rats received drinking water contain 30 ppm cadmium and intraperitoneally injected with 50 mg/kg/day CaNa$_2$EDTA macroparticles or nanoparticles at the end of the 6th till the 10th week of the experiment for 4 courses (4 days each) with 3 days interval between the courses

Groups / Time	Control	Cadmium	Cadmium & CaNa$_2$EDTA Macroparticles	Cadmium & CaNa$_2$EDTA Nanoparticles
2nd week	31.4 ± 0.8 a	37.6 ± 0.7 b		
4th week	32.3 ± 1.3 a	40.9 ± 1.4 b		
6th week*	33.4 ± 1.9 a	44.2 ± 1.2 b		
8th week	34.9 ± 1.3 a	43.6 ± 2.0 b	40.5 ± 1.4 b	41.1 ± 1.8 b
10th week	31.6 ± 1.6 a	45.0 ± 1.0 b	38.5 ± 1.2 c	32.8 ± 1.8 a

Values have different scripts at the same row are significantly different at P ≤ 0.05 (n=10)

*Start of the treatment with CaNa$_2$EDTA macroparticles or nanoparticles.

Serum Creatinine Concentration:

Cadmium group showed a significant increase in their serum creatinine concentration from the control group. This toxic effect was largely ameliorated by CaNa$_2$EDTA nanoparticles treatment as rats in this group showed an insignificant increase from the control group and a significant decrease than those of the other experimental groups. In contrast, CaNa$_2$EDTA macroparticles group showed a significant increase in serum creatinine concentration when compared to the control group (Table 3).

Table (3): Mean values ±S.E of the serum creatinine concentration (mg/dl) in control and experimental rats received drinking water contain 30 ppm cadmium and intraperitoneally injected with 50 mg/kg/day CaNa$_2$EDTA macroparticles or nanoparticles at the end of the 6th till the 10th week of the experiment for 4 courses (4 days each) with 3 days interval between the courses.

Groups\Time	Control	Cadmium	Cadmium and CaNa$_2$EDTA Macroparticles	Cadmium and CaNa$_2$EDTA Nanoparticles
2nd week	0.9 ± 0.05 a	1.2 ± 0.07 b		
4th week	1.0 ± 0.04 a	1.2 ± 0.05 b		
6th week*	0.9 ± 0.05 a	1.9 ± 0.03 b		
8th week	0.7 ± 0.03 a	2.8 ± 0.09 b	1.6 ± 0.06 c	0.8 ± 0.02 a
10th week	0.7 ± 0.04 a	5.4 ± 0.09 b	1.2 ± 0.07 c	0.8 ± 0.03 a

Values have different scripts at the same row are significantly different at P ≤ 0.05 (n=10)

*Start of the treatment with CaNa$_2$EDTA macroparticles or nanoparticles.

Serum Cadmium Concentration:

Cadmium was not detected in the serum of the control group along the entire period of the experiment. Serum cadmium concentration of the cadmium group showed a gradual increase throughout the experiment. This increase was modulated by CaNa$_2$EDTA treatment with special reference to the nanoparticles form, which showed a significant decrease at the 8th and 10th weeks of the experiment, while the macroparticles form showed this decrease only at the 10th week of the experiment (Table 4).

Table (4): Mean values ± S.E of the serum cadmium concentration (ppb) in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50mg/kg/day CaNa$_2$EDTA macroparticles or nanoparticles at the end of the 6th till the 10th week of the experiment for 4 courses (4 days each) with 3 days interval between the courses.

Time \ Groups	Control	Cadmium	Cadmium & CaNa$_2$EDTA Macroparticles	Cadmium & CaNa$_2$EDTA Nanoparticles
2nd week	ND	12.2 ± 0.6 a		
4th week	ND	12.8 ± 0.6 a		
6th week*	ND	13.8 ± 0.5 a		
8th week	ND	14.6 ± 1.2 a	12.96 ± 1.4 ab	10.04 ± 1.0 b
10th week	ND	14.3 ± 1.3 a	10.82 ± 1.1 b	8.02 ± 0.75 b

Values have different scripts at the same row are significantly different at P ≤ 0.05 (n=10)

*Start of the treatment with CaNa$_2$EDTA macroparticles or nanoparticles

ND: not detected; less than 1 ppb

Urine Cadmium Concentration:

Cadmium was not detected in the urine of the control group along the entire period of the experiment. Urine cadmium concentration of the cadmium group showed a gradual increase throughout the experiment. Cadmium excretion in urine was largely enhanced by CaNa$_2$EDTA nanoparticles treatment, which showed a significant increase at the 8th and 10th weeks of the experiment, while the macroparticles form of CaNa$_2$EDTA showed this significant increase only at the 8th week of the experiment (Table 5).

Table (5): Mean values ± S.E of the urine cadmium concentration (ppb) in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50mg/kg/dayCaNa₂EDTA macroparticles or nanoparticles at the end of the 6th till the 10th week of the experiment for 4 courses (4 days each) with 3 days interval between the courses.

Groups / Time	Control	Cadmium		
2nd week	ND	15.7 ± 0.44 a		
4th week	ND	15.8 ± 0.34 a		
6th week*	ND	16.0 ± 0.52 a	Cadmium and CaNa₂EDTA Macroparticles	Cadmium and CaNa₂EDTA Nanoparticles
8th week	ND	16.4 ± 0.51 a	18.9 ± 0.51 b	19.3 ± 0.66 b
10th week	ND	16.3 ± 0.68 a	18.0 ± 0.73 ab	19.7 ± 0.86 b

Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n=10)

*Start of the treatment with CaNa₂EDTA macroparticles or nanoparticles

ND: not detected; less than 1 ppb.

Histopathological Findings:

Microscopic examination of tissue prepared sections from liver, kidney and femur of control rats revealed normal histological structure. Regarding livers of cadmium administrated rats revealed histological alterations; the hepatic cells appeared greatly swollen with granular and vacuolar degeneration as well as many necrotic cells especially in the centrilobular area and extended peripherally Fig.(1A). Activated Kupffer cells were apparent near the hepatocellular necrobiotic changes. The vacuolar degeneration was few, scattered and of microvesiculartype Fig.(1B).Liver of cadmium intoxicated and CaNa₂EDTA macroparticles treated rats' revealed moderate restoration of the hepatic parenchymal cells, but there were still cellular swelling and necrobiotic changes of moderate intensity. However, livers of cadmium intoxicated and CaNa₂EDTA nanoparticles treated rats' revealed marked restoration of the hepatic parenchymal cells almost near to normal with only very few hepatic cells appeared with granular degeneration and scarcely necrotic cells.

Kidney of cadmium-intoxicated rats showed severe degree of degenerative changes of the

renal tubular epithelial linings of granular and vacuolar types with many necrotic and desquamated cells Fig. (1C). Most of the necrotic cells were desquamated in the tubular lumens, showed presence of granular cast. The intertubular blood vessels were congested. The renal glomeruli were severely affected, most of them showed hypercellularity of their glomerular tufts with thickening of both the glomerular basement membrane and the parietal layer of Bowman's capsule Fig. (1D). While kidney of cadmium-administrated rats and treated with CaNa$_2$EDTA macroparticles showed mild to moderate degenerative changes of the renal tubular epithelium with scattered necrotic cells, few desquamated cells and some renal casts in the lumen of some tubules. Some renal glomeruli showed vacuolation of the podocytes. Concerning kidney of CaNa$_2$EDTA nanoparticles treated rats after cadmium intoxication revealed good restoration of the renal glomeruli with only mild necrobiotic changes of the renal tubular epithelium and appearance of regenerated foci in the tubules. Regarding microscopic examination of femur diaphysis for cadmium treated rats showed variable sizes areas of bone erosions, resorption and appearance of bony spicules Fig. (1E) with decreased density of the collagen fibers. The observed areas of bone resorption were accompanied with many osteoclast cells inside the resorbed areas; the osteocytes in the vicinity were present in their lacunae Fig. (1F). However, examination of different sections of femur of cadmium administrated rats and treated with CaNa$_2$EDTA macroparticles showed some areas of bone resorption surrounded with active dark lines of bone deposition. Cross section of femur diaphysis compact bone of cadmium administrated rats and treated with CaNa$_2$EDTA nanoparticles showed that, the osteocytes were present inside their lacunae, osteoclast cell inside a small area of bone resorption surrounded with dark lines of bone deposition and re-deposition of collagen. Marked dark lines of bone deposition were conspicuously observed with regularly and tightly arranged collagen fibers.
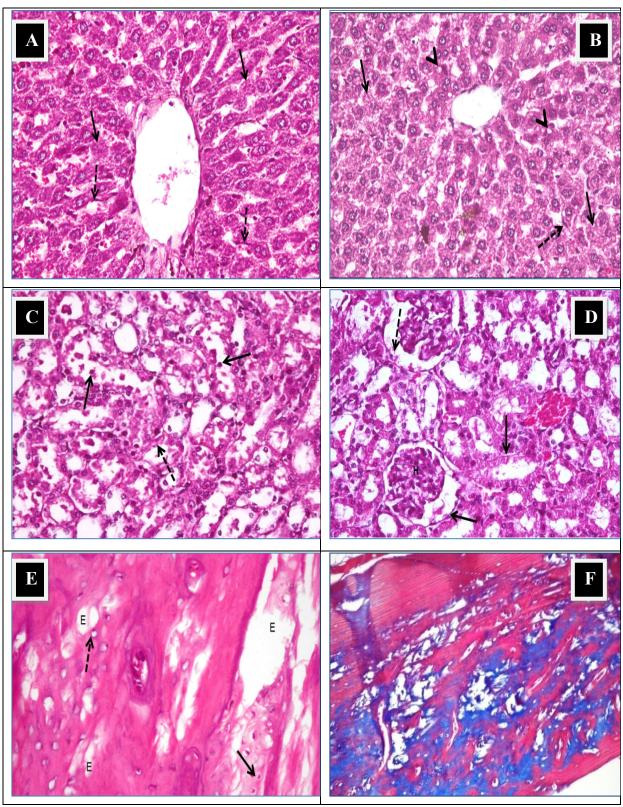
Fig. (1): (A): Liver of cadmium-administrated rat showing swelling of the hepatic cells, degeneration and necrosis (arrow) of many cells especially in the centrilobular area as well as activated Kupffer cells (dashed arrow). **(H&E, X400), (B):** liver of cadmium administrated

rat showing hepatocellular swelling, granular and few vacuolar degeneration (arrow head), many necrotic cells (arrow) as well as activated Kupffer cells (dashed arrow). **(H&E, X400), (C):** kidney of cadmium administrated rat showing granular and vacuolar degeneration of the renal tubular epithelium with many necrotic (dashed arrow) and desquamated cells (arrow), **(H&E, X400). (D):** kidney of cadmium administrated rat showing hypercellularity of the glomerular tuft **(H),** thickening of both the glomerular basement membrane and the parietal layer of Bowman's capsule (arrow) as well as presence of granular cast in the Bowman's space (dashed arrow), **(H&E, X400). (E):** cross section of femur diaphysis compact bone of cadmium administrated rat showing areas of bone resorption with many osteoclast cells inside the resorbed areas with many areas of bone erosions **(E)** with many osteoclast (arrow) and osteocytes in their lacunae (dashed arrow), **(H&E, X400). (F):** cross section of femur diaphysis compact bone of cadmium- intoxicated rat showing areas of bone resorption and decreased collagen fiber staining the bone lamellae; Masson's trichrome stain **(X400).**

DISCUSSION

Cadmium poisoning is reported in many parts of the world. It is one of the global health problems affect many organs. Long-term exposure to cadmium through air, water, soil, and food leads to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous, and respiratory systems **(Rafati *et al.,* 2017).** In the current study, LD_{50} value of cadmium sulfate in adult female Sprague Dawley rats was 240 mg/kg. **Lewis, (2004),** has reported similar value of **LD50 and Lide, (2006)** they reported that, the oral LD_{50} value of cadmium sulfate in rats is 280mg/kg. **USAF, (1990)** recorded that, the oral LD_{50} values of cadmium in animals range from 63 to 1125 mg/kg, depending on the cadmium compound.

Rats received drinking water contain 30-ppm cadmium showed marked depression. **Jafarpour *et al.,*(2017),**observed similar symptoms**.** This depression was slightly ameliorated by CaNa2EDTA macroparticles treatment, and largely ameliorated with CaNa2EDTA nanoparticles treatment. Frequent urination was observed in CaNa2EDTA macroparticles and nanoparticles treated rats because of EDTA treatment **(Bethesda, 2011).** Growth inhibition is the general signs of Cd exposure **(Choi and Rhee, 2001).** In the present study, cadmium intoxicated rats had significantly low body weight. The adverse effect of cadmium on growth performance could be related to affecting nutritional absorption and metabolism and reducing serum proteins. **Tinkov *et al.,* (2018)** recorded that Cd exposure induces a significant

alteration of bacterial populations and their relative abundance in gut, accompanied by increased lipopolysaccharide (LPS) production, reflecting changed metabolic activity of the intestinal microflora. **(Gaurav *et al.,* 2010; Nasim *et al.,* 2015 and Jafarpour *et al.,* 2017)** have reported similar decreased body weight**.** Treatment with CaNa$_2$EDTA nanoparticles offered a more pronounced protection against cadmium toxicity than CaNa$_2$EDTA macroparticles, which could be attributed to a more powerful chelating capacity to cadmium and consequently less toxicity and more improved performance parameters.

Cadmium intoxicated rats showed a significant increase in serum urea and creatinine concentrations when compared with normal control group. These results came in accordance with the recorded data of **Samy *et al.,* (2014); El-Boshy *et al.,* (2015) and Jafarpour *et al.,* (2017).** The increase of serum urea and creatinine concentrations in cadmium-exposed rats may be attributed to the toxic effect of cadmium on the renal tubules and glomeruli leading to nephrotoxicity and renal tubular damage **(Aisha and Elham, 2000).**Treatment with CaNa$_2$EDTA macroparticles showed a marked improvement in kidney parameters, while kidney parameters of rats treated with CaNa$_2$EDTA nanoparticles were near normal when compared to the control group. These findings suggest that treatment with CaNa$_2$EDTA macroparticles or nanoparticles alleviate the nephrotoxic effect of cadmium with special reference to the nanoparticles form, which provides a more powerful cadmium chelating capacity, and thus less toxic effects of cadmium. Serum cadmium concentration and urinary cadmium excretion were significantly increased in cadmium-intoxicated rats. These results are consistent with other studies **(Shim, 2008 and Kim *et al.,* 2009).** Rats treated with CaNa$_2$EDTA macroparticles or nanoparticles showed relatively lower serum cadmium concentrations and relatively higher urine cadmium concentrations when compared to the cadmium intoxicated rats. These findings could be attributed to cadmium chelation by CaNa$_2$EDTA macroparticles or nanoparticles and enhanced excretion. These effects were more pronounced in CaNa$_2$EDTA nanoparticles treated rats. Increased urinary cadmium losses by EDTA therapy has also reported by **Waters *et al.,* (2001).**Treatment with CaNa$_2$EDTA macro or nanoparticles lowered the toxic effect of cadmium on the liver, kidney and femur especially the nanoparticles form as shown on histopathological examination. The liver is the primary target organ following acute systemic cadmium exposure. In the present work, the administration of cadmium resulted in degeneration and necrosis of

hepatocytes. These findings are in concurrence with **Randa *et al.,* (2012) and El-Refaiy and Eissa, (2013).** The histopathological changes of the liver treated with cadmium might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by cadmium **(Renugadevi and Prabu, 2010)**. Severe degenerative changes of the renal tubular epithelial linings with many necrotic and desquamated cells were observed after treatment with cadmium in the present work. Marked inter-tubuar hemorrhages in the renal medulla with necrobitic changes of the medullary tubular epithelium and focal mononuclear inflammatory cells infiltration in the interstitial tissue and at the renal pelvis with marked pyelitis in some rats were also observed. Similar findings have been reported by **Jemai *et al.,* (2010); Randa *et al.,* (2012) and El-Refaiy and Eissa, (2013).** Cadmium-induced nephrotoxicity is thought to be mediated through the cadmium metallothionein complex, which is synthesized in the liver, released into circulation and taken up by renal proximal tubule cells **(Dudley *et al.,* 1985).** In fact, when the synthesis of metallothionein becomes insufficient for binding all cadmium ions in the liver, cadmium not bound to metallothionein produces hepatocyte injury and a cadmium metallothionein complex is released into the blood stream. The complex in the plasma is then, filtered through the glomeruli in the kidney and taken up by the proximal tubular cells **(Sudo *et al.,* 1996).** On its way through the kidney, this complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function **(Thijssen *et al.,* 2007).** Moreover, these changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free cadmium ions in the renal tissues of cadmium -treated rats **(Renugadevi and Prabu, 2009).** In the present study, cadmium intoxicated rats showed bone erosions, bone resorption with many osteoclast cells inside the resorbed areas, bony spicules and decreased density of the collagen fibers. Several studies in workers exposed to cadmium-polluted fume and dust showed a connection between cadmium intoxication and bone damage **(Kazantzis, 1979).** Cadmium toxicity is associated with the occurrences of Itai-Itai, a disease under which patients show a wide range of symptoms such as low grade of bone mineralization, high rate of fractures, increased rate of osteoporosis, and intense bone associated pain. Mechanisms of Cd toxicity in bone include stimulation of fibroblast growth factor, which induces phosphaturia and decreases phosphate uptake, leading to osteomalacia **(Kido *et al.,* 2012).** Cd is toxic to MC3T3 osteoblasts by unknown mechanisms **(Lizotte *et al.,* 2012)** and stimulates osteoclasts, thereby inducing osteoporosis **(Chen *et al.,* 2012).** Cd decreases serum

osteocalcin levels in rats **(Youness *et al.,* 2012).** These factors apparently combine to induce calciuria, increase bone resorption and decrease bone mineral density in Cd-exposed children **(Sughis *et al.,* 2011).** The role of CaNa$_2$EDTA nanoparticles could be due to reduction in size, and difference in shape of the nanoformulation, which is evenly spherical because of precipitation process, as compared to irregular shape of the micronized forms.This is resulting from machining or grinding processes, which provide an optimization to their use as a chelating agent for cadmium toxicity and thus minimizing its toxic effects **(Kumar *et al.,* 2011).** In conclusion, this study recommends that CaNa$_2$EDTA 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.

REFERENCES

Aisha, M. F. and Elham, A. M. (2000): Interaction of iron, zinc and calcium with cadmium toxicity in rats and goats. J. Egypt Vet. Med. Ass., 60: 203 - 218.

Andersen, O.; Nielsen, J. B. and Svendsen, P. (1988): Oral cadmium chloride intoxication in mice: effects of chelation. Toxicology, 52 (1-2): 65-79.

Bethesda, M. D. (2011): American Society of Health-System Pharmacists. AHFS Drug Information, 1st Edition.

Borenfreund, E. and Puerner, J. A. (1986): Cytotoxicity of metals, metal-metal and metal-chelator combinations assayed in vitro. Toxicology, 39 (2): 121-134.

Chen, X.; Zhu, G.; Jin, T.; Zhou, Z.; GU; Qiu, J. and Xiao, H. (2012): Cadmium stimulates the osteoclastic differentiation of RAW264.7 cells in presence of osteoblasts. Biol. Trace Elem. Res., 146 (3): 349-353.

Choi, J. H. and Rhee, S. J. (2001): Effects of green tea catechin on cadmium accumulation in chronic cadmium poisoned rats. Korean J. Nutr., 34: 384 -392.

Donald, B. C.; Edward, A. and Ashwood, A. D. (1996): Tiezts- Fundermental Clinical Chemistry, 416: 145 - 155.

Dudley, R. E.; Gammal, L. M. and Klaassen, C. D. (1985): Cadmium induced hepatic and renal injury in chronically exposed rats: likely role of hepatic cadmium-metallothionein in nephrotoxicity. Toxicol. Appl. Pharmacol., 77 (3): 414 - 426.

El-Boshy, M. E.; Risha, E. F.; Abdel - Hamid, F. M.; Mubarak, M. S. and Hadda, T. B. (2015): Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. J. Trace Elem. Med. Biol., 29:104-110.

El-Refaiy, A. I. and Eissa, F. I. (2013): Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi. J. Biol. Sci., 20 (3): 265-280.

El-Shehawi, A. M.; Ali, F. K. and Seehy, M. A. (2007): Estimation of water pollution by genetic biomarkers in tilapia and catfish species shows species-site interaction. Afr. J. Biotechnol., 6 (7): 840-846.

Falk, R. T.; Pickle, L. W.; Fontham, E. T.; Correa, P.; Morse, A.; Chen, V. and Fraumeni, JJ. Jr. (1990): Occupation and pancreatic cancer risk in Louisiana. Am. J. Ind. Med., 18 (5): 565-576.

Fischer, Y. W. (2008): Manual of Stroke Models in Rats. Published by Taylor Francis (CRC Press). 59, 1- 352.

Foreman, H. (1961): Use of chelating agents in treatment of metal poisoning (with special emphasis on lead). Fed. Proc., 20 (3): 191-196.

Fortina, P., Kricka, L. J.; Surrey, S. and Grodzinski, P. (2005): Nanobiotechnology: the promise and reality of new approaches to molecular recognition. Trends Biotechnol, 23(4): 168-173.

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

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

Graig, D. S. and Wayne, C. (1984): Simple automated wet digestion of animal tissues for determination of seven elements by atomic absorption spectroscopy. Tec. Com., 107:115.

Ibim, S. E.; Trotman, J.; Musey, P. I. and Semafuko, W. E. (1992): Depletion of essential elements by calcium disodium EDTA treatment in the dog. Toxicol, 73: 229-237.

Jafarpour, D.; Shekarforoush, S. S.; Ghaisari, H. R.; Nazifi, S.; Sajedianfard, J. and Eskandari, M. H. (2017): Protective effects of synbiotic diets of *Bacillus coagulans*, *Lactobacillus plantarum* and inulin against acute cadmium toxicity in rats. BMC Complement Altern. Med., 17: 291.

Jain, K. K. (2005): The role of nanobiotechnology in drug discovery. Drug Discov. Today, 10 (21): 1435-1442.

Järup, L.; Hellström, L.; Alfvén, T.; Carlsson, M. D.; Grubb, A.; Persson, B.; Pettersson, C.; Spång, G.; Schütz, A. and Elinder, C. (2000): Low level exposure to cadmium and early kidney damage: the OSCAR study. Occup. Environ. Med., 57 (10): 668-672.

Jemai, H.; Lachkar, H. A.; Messaoudi, I. and Kerkeni, A. (2010): Effect of zinc pre-treatment on blood glutathione, serum zinc and kidney histological organisation in male rats exposed to cadmium. J. Trace Elem. Med. Biol., 24 (4), 277–282.

Kazantzis, G. (1979): Renal tubular dysfunction and abnormalities of calcium metabolism in cadmium workers. Environ. Health Perspect, 28:155-159.

Kido, S.; Fujihara, M.; Nomura, K.; Sasaki, S.; Shiozaki, Y.; Segawa, H.; Tatsumi, S. and Miyamoto, K. (2012): Fibroblast growth factor 23 mediates the phosphaturic effect of cadmium. Nihon EiselgakuZasshi, 67(4):464-471.

Kieman, J. A. (1999): Histological and Histochemical methods. Theory and Practice (3rd ed) Butterworth Heinemann, Oxford, Boston, New Dehli.

Kim, Y. J.; Kwon, S. and Kim, M. K. (2009): Effect of Chlorella vulgaris intake on cadmium detoxification in rats fed cadmium. Nutr. Res. Pract., 3(2):89-94.

Klaassen, C. D.; Liu, J. and Choudhuri, S. (1999): Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol, 39:267-294.

Knudtson, M. L.; Wyse, K. G. and Galbraith, P. D. (2002): Chelation therapy for ischemic heart disease. A randomized controlled trail. JAMA, 287: 481- 486.

Kumar, N.; Soni, S.; Jaimini, A.; Ahmad, F. J.; Bhatnagar, A. and Mittal, G.(2011): Edetate calcium disodium nanoparticle dry powder inhalation: a novel approach against heavy metal decorporation. Int. J. Pharm., 416(1):376-383.

Lafuente, A.; Márquez, N.; Pérez-Lorenzo, M.; Pazo, D. and Esquifino, A. I. (2000): Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary- testicular axis function in the rat. Food Chem. Toxicol., 38 (10): 913 - 923.

Lewis, R. J. (2004): Sax's Dangerous Properties of Industrial Materials, 11th Edition. Wiley-Interscience, Wiley and Sons, Inc. Hoboken, NJ, p. 663.

Lide, D.R. (2006): CRC Handbook of Chemistry and Physics, 87th ed., Boca Raton, FL: CRC Press. ISBN 0-8493-0487-3.

Lizotte, J.; Abed, E.; Signor, C.; Malu, D. T.; Cuevas, J.; Kevorkova, O.; Sanchez-Dardon, J.; Satoskar, A.; Scorza,T.; Jumarie, C.and Moreau, R. (2012): Expression of macrophage migration inhibitory factor by osteoblastic cells: protection against cadmium toxicity. Toxicol. Lett, 215(3): 167-173.

Llobet, J. M.; Granero, S.; Torres, A.; Schumacher, M. and Domingo, J. L. (1998): Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain III. Blood levels. Trace Elem. Electr., 15: 76-80.

Matović, V.; Buha, A.; Bulat, Z. and Dukić-Ćosić, D. (2011): Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. Arh. Hig. Rada. Toksikol, 62 (1):65-76.

Nasim, B.; Ali, A.; Kahin, S. and Somaye, B. (2015): The protective roles of zinc and magnesium in cadmium-induced renal toxicity in male wistar rats. Iran. J. Toxicol., 8 (27):1160 -1167.

Rafati, R. M.; Rafati, R. M.; Kazemi, S. and Moghadamnia, A. A. (2017): Cadmium toxicity and treatment: An update. Caspian J Intern Med., 8(3): 135-145.

Randa, A. H.; Dawlat, M. A.; Nariman, A. R.; Hatem, M. E. and Dessouky, M. I. (2012): Clinicopathological, histopathological and immunolog1ical studies on animals exposed to lead and cadmium under experimental conditions. N. Y. Sci. J., 5(12): 120-136.

Renugadevi, J. and Prabu, S. M. (2009): Naringenin protects against cadmium-induced renal dysfunction in rats. Toxicology, 256 (1-2): 128-134.

Renugadevi, J. and Prabu, S. M. (2010): Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp. Toxicol. Pathol, 62 (2): 171-181.

Samy, A. H.; Omnia, M. A. and Ahmad, M. S. (2014): Protective effects of alpha-lipoic acid and melatonin against cadmium-induced oxidative stress in erythrocytes of rats. Benha Vet. Med. J., 26 (1): 19-33.

Shim, J. A. (2008): Effect of Chlorella intake on Cadmium metabolism in rats. Master thesis. Ewha Womans University, Seoul. Republic of Korea.

Shimada, H.; Funakoshi, T. and Waalkes, M. P. (2000): Acute, Nontoxic Cadmium Exposure Inhibits Pancreatic Protease Activities in the Mouse. Toxicol. Sci., 53(2): 474 - 480.

Sudo, J.; Hayashi, T.; Kimura, S.; Kakuno, K.; Terui, J.; Takashima, K. and Soyama, M. (1996): Mechanism of nephrotoxicity induced by repeated administration of cadmium chloride in rats. J. Toxicol. Environ. Health, 48 (4): 333-348.

Sughis, M.; Penders, J.; Haufriod, V.; Nemery, B. and Nawrot, T. S. (2011): Bone resorption and environmental exposure to cadmium in children: a cross-sectional study. Environ. Health, 10: 104.

Thijssen, S.; Maringwa, J.; Faes, C.; Lambrichts, I. and Kerkhove, E. V. (2007): Chronic exposure of mice to environmentally relevant, low doses of cadmium leads to early renal damage, not predicted by blood or urine cadmium levels. Toxicology, 229 (1-2):145-156.

Tinkov, A. A.; Gritsenko, V. A.; Skalnaya, M. G.; Cherkasov, S. V.; Aaseth, J. and Skalny, A. V. (2018): Gut as a target for cadmium toxicity. Environmental Pollution, 235: 429-434.

Turner, R. (1965): Quantal responses. Calculation of ED50. In Screening Methods in Pharmacology, Academic Press, New York, 61-63.

USAF (1990): Cadmium. In: Installation Restoration Program Toxicology Guide. Vol.5. Wright Patterson AFB, OH: Harry G. Armstrong Aerospace Medical Research Laboratory.

Vassault, A.; Grafmeyer, D. and Naudin (1986): Protocole de validation de techniques. Ann. Biol. Clin., 44:686.

Waalkes, M. P.; Anver, M. R. and Diwan, B. A. (1999): Chronic toxic and carcinogenic effects of oral cadmium in the Noble (NBL/Cr) rat: Induction of neoplastic and proliferative lesions of the adrenal, kidney, prostate, and testes. J. Toxicol. Environ. Health, 58 (4): 199-214.

Waters, R. S.; Bryden, N.A.; Patterson, K. Y.; Veillon, C. and Anderson, R. A. (2001): EDTA chelation effects on urinary losses of cadmium, calcium, chromium, cobalt, copper, lead, magnesium, and zinc. Biol. Trace Elem. Res., 83(3):207-221.

Youness, E. R.; Mohammed, N. A. and Morsy, F. A. (2012): Cadmium impact and osteoporosis: mechanism of action. Toxicol. Mech. Methods, 22 (7):560-567.

Young, D. S.; Pestaner, L. C. and Gibberman, V. (1975): Effect of drugs on laboratory tests. Clin Chem., 21: 286-288.