j.Egypt.vet.med.Assoc 78, no 3. 427– 441 (2018)

IMMUNOTOXICITY OF CADMIUM IN FEMALE RATS AND THE AMELIORATIVE EFFECT OF CALCIUM DISODIUM EDTA NANOPARTICLES

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.

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

ABSTRACT

Cadmium is very toxic substances due to its wide range of organ toxicity and long elimination half-life of 10-30 years. EDTA is the agent most widely accepted for clinical treatment of cadmium toxicity. Nanoparticulate drug delivery systems are used to achieve a more effective treatment and minimizing side effects**.** This study was concerned with the study of the immunotoxic effects of cadmium on adult female rats as well as the ameliorating effects of $CaNa_2EDTA$ nanoparticles against such toxicity. The predictive protective effect was compared with $CaNa_2EDTA$ macroparticles. At the start of the experiment, animals were divided into two groups; one acted as control (contains15 rats) and the other one (contains15 rats) received drinking water contains 30 ppm cadmium for 10 weeks. At the end of the 6^{th} week of the experiment, the cadmium-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 cellular immune response (as determined by assessment of total and differential leucocytic count and phagocytic activity of neutrophils) was significantly reduced by cadmium. Lymphocytes percentage was also decreased while neutrophils and monocytes percentages were increased. Humoral immune response as estimated by the measurement of electrophoresis pattern of serum proteins (albumin, alpha, beta and gamma globulins) reported a significant inhibition in cadmium intoxicated rats except levels of beta globulins, which showed a significant increase. The alterations that associated with cadmium toxicity were markedly alleviated by $CaNa_2EDTA$ nanoparticles treatment,while $CaNa_2EDTA$ macroparticles treatment induced a mild protective effect against cadmium toxicity when compared to 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 immunotoxic effects of cadmium in rats.

Keywords:

Cadmium, Immunotoxicity, $CaNa_2EDTA$, Nanoparticles, Female rats.

INTRODUCTION

Cadmium (Cd) is a naturally occurring metal situated in the Periodic Table of the Elements between zinc and mercury, with chemical behavior similar to zinc. Cadmium is a transitional metal that exists in different oxidational or transitional states **(Donald *et al.,* 1996).** 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 **(U. S. Geological Survey, 2012).**

Cadmium exposure occurs from ingestion of contaminated food (e.g., crustaceans, leafy vegetables, rice from certain areas of Japan and China) or water can produce long-term health effects. Contamination of drugs and dietary supplements may also be a source of contamination **(Abernethy *et al.,* 2010).** Cadmium has varying degrees of solubility, absorption and toxicity **(Falk *et al.,* 1990).**

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).** Cadmium causes mutations, deoxyribonucleic acid (DNA) strand breaks, chromosomal damage, cell transformation and impaired DNA repair in cultured mammalian cells **(NTP, 2004).** Cadmium modulates gene expression and signal transduction **(Waisberg *et al.,* 2003).** 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).** 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 **(Hanson *et al.,* 2010 and 2012).** Post-natal Cd exposures induce cell cycle arrest and apoptosis in splenocytes **(Chatterjee *et al.,* 2009).** Cd induces increased rates of autoimmunity, increased production of nonspecific antibodies,

and decreased production of antigen-specific antibodies **(Ohsawa,2009).**Cd also suppressed lymphocyte proliferation and natural killer cell activity **(Fortier *et al.,* 2008).** Several chelators have been 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, and is used seldom 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 principal and most serious toxic effect of calcium disodium edetate ($CaNa_2EDTA$) is renal tubular necrosis, which tends to occur when the daily dose is excessive and may result in fatal nephrosis. Calcium disodium edetate may produce the same signs of renal damage as lead poisoning, such as proteinuria and microscopic hematuria. Rarely, changes in distal renal tubules and glomeruli, glycosuria, presence of large renal epithelial cells in urinary sediment, increased urinary frequency, and urgency may occur. Hydropic degeneration of proximal renal tubular cells also may occur; however,recovery usually occurs following discontinuance of therapy **(Bethesda, 2011).** Prolonged treatment with $CaNa_2EDTA$ results in depletion of essential metal, especially Zinc, Copper and Manganese **(Ibim *et al.,* 1992)**. 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 **(Fortina *et al.,* 2005)**. 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 **(Jain, 2005).**

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

MATERIAL AND METHODS

Animals:

Sixty adult female Sprague Dawley rats weighing (150 ± 15 g) were used for the toxicological assessment. They were obtained from the Department of Behavior, Faculty of Veterinary Medicine, and Cairo University, Egypt. The animals were kept under observation in the animal house 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).

Chemicals and drugs:

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 30 ppm 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 four 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. $CaNa_2EDTA$ 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 CaNa2EDTAwas prepared by nanoprecipitation method.$CaNa_2EDTA$ 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).**

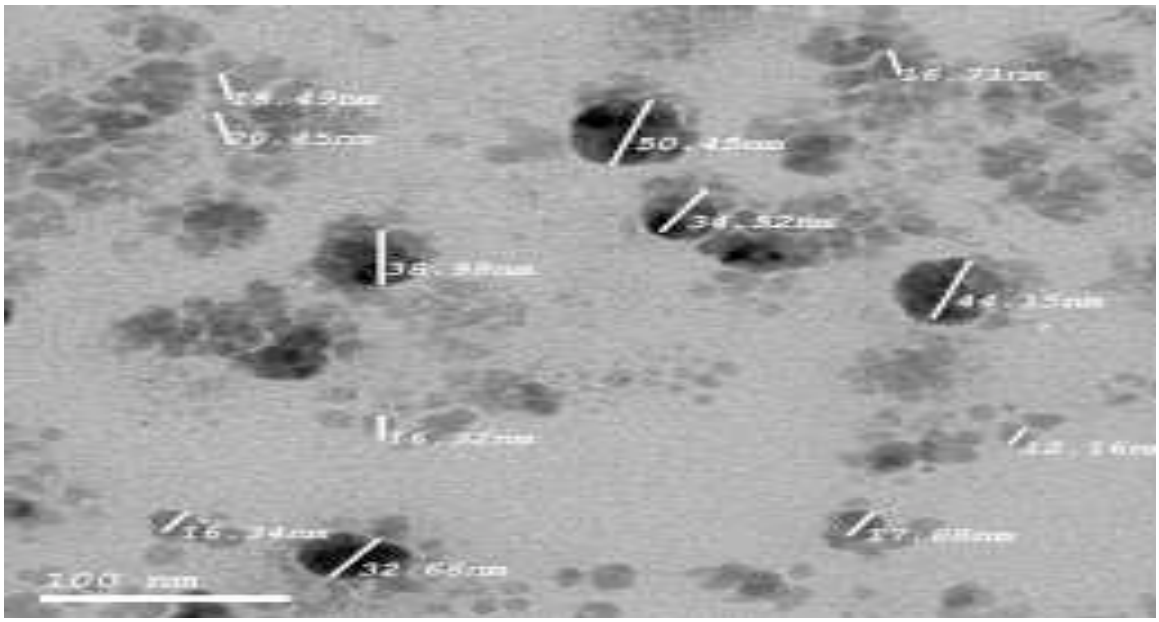

Fig. (1): Transmission electron micrograph for $CaNa_2EDTA$ nanoparticles.

Experimental Design:

At the start of the experiment, animals were classified into two groups; a control (contains 15 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 $CaNa_2EDTA$ for four courses (4 days each) with an interval of 3 days between the courses. Every two weeks, ten animals from each group were randomly selected and blood samples were collected from the inner census of the eye. At the end of the experiment, all animals were sacrificed. Animals were anesthetized by intra-peritoneal injection of 100 mg/kg ketamine hydrochloride **(Fischer, 2008)** followed by decapitation. Three types of blood samples were collected every two weeks from ten animals per each group. The first samples were collected without addition of anticoagulant for serum separation. The second samples were collected with EDTA for estimation of total and differential leucocytic counts. The third samples were collected with heparin for assessment of phagocytic activities of the neutrophils. Total and differential leucocytic counts were determined according to **Schalm *et al.*, (1975).** Estimation of phagocytic activity of neutrophils was applied using Nitro blue tetrazolium dye (NBT) and Zymosan according to the method described by **Gifford and Malawista, (1970).** Serum protein fractions of the

collected blood samples were determined using electrophoresis pattern sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by **Wang and Hurley, (1998).** 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

Total Leucocytic Count

The addition of cadmium to the drinking water of rats resulted in a significant decrease in their total WBCs count compared with those of the control group. Contrariwise, rats intoxicated by cadmium and treated with $CaNa_2EDTA$ nanoparticles restored this decrease to a large degree with counts close to those of the control group, while counts of rats treated with $CaNa_2EDTA$ nanoparticles were near those of the cadmium group (Table 1).

Table (1): Mean values ± S.E of the total leucocytic count($10^3/\mu l$) in control and experimental rats received drinking water contain 30ppmcadmium and intraperitoneally injected with 50mg/kg/day $CaNa_2EDTA$ 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	**10.7 ± 0.7 a**	**8.3 ± 0.4 b**		
4th week	**11.5 ± 0.8 a**	**9.6 ± 0.5 b**		
6th week*	**9.5 ± 0.6 a**	**7.3 ± 0.5 b**	**Cadmium and $CaNa_2EDTA$ Macroparticles**	**Cadmium and $CaNa_2EDTA$ Nanoparticles**
8th week	**8.6 ± 0.8 a**	**6.6 ± 0.4 b**	**7.6 ± 0.4 abc**	**8.8 ± 0.9 ac**
10th week	**8.4 ± 0.6 a**	**7.6 ± 0.3 b**	**7.7 ± 0.6 ab**	**8.2 ± 0.2 ab**

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

***Start of the treatment with $CaNa_2EDTA$ macroparticles or nanoparticles**

Differential Leucocytic Count:

Leucocytic cells were differentiated into lymphocytes, neutrophils, monocytes, eosinophils and basophils. Each type of cells was expressed as a percentage of the total leucocytic count. The percentages of both eosinophils and basophiles were about 2% and there was not a difference found between the control and experimental groups in their percentage. Cadmium intoxicated rats showed a significant decrease in their lymphocytes percentage (Table 2) along the entire experiment and a significant increase in their neutrophils percentage (Table 3) except at the 4th and 6th week of the experiment when compared to the control rats**.** However, treatment with $CaNa_2EDTA$ macroparticles or nanoparticles significantly elevated the decrease in lymphocytes percentage and significantly reduced the increase in neutrophils percentage when compared to the cadmium group with special reference to the nanoparticles form especially at the last week of the experiment as lymphocytes and neutrophils percentages were very close to those of the control group. Monocytes percentage of the cadmium and other experimental groups (Table 4) showed insignificant increase from those of the control group along the whole length of the experiment with the exception of the $CaNa_2EDTA$ nanoparticles group, which showed a significant difference when compared to the control and the cadmium groups at the 8th week of the experiment.

Table (2): Mean values ± S.E of the lymphocytes percentage in control and experimental rats received drinking water contain 30 ppm cadmium and intraperitoneally injected with 50mg/kg/day $CaNa_2EDTA$ 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	51.1 ± 1.3 a	38.3 ± 0.5 b		
4th week	37.9 ± 1.0 a	32.8 ± 1.2 b		
6th week*	46.2 ± 2.2 a	38.9 ± 2.0 b	Cadmium and $CaNa_2EDTA$ Macroparticles	Cadmium and $CaNa_2EDTA$ Nanoparticles
8th week	44.7 ± 1.4 a	34.0 ± 1.4 b	40.7 ± 2.6 a	39.8 ± 1.6 a
10th week	42.2 ± 1.7 a	31.8 ± 1.1 b	40.3 ± 1.3 a	40.7 ± 1.7 a

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

***Start of the treatment with $CaNa_2EDTA$ macroparticles or nanoparticles.**

Table(3): Meanvalues and±S.E of the neutrophils percentage in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50mg/kg/day $CaNa_2EDTA$ 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	44.1 ± 1.2 a	56.3 ± 0.4 b		
4th week	56.3 ± 1.1 a	60.9 ± 2.2 a		
6th week*	49.3± 2.3 a	55.4 ± 2.0 a	Cadmium and $CaNa_2EDTA$ Macroparticles	Cadmium and $CaNa_2EDTA$ Nanoparticles
8th week	51.5 ± 1.4 a	61.9 ± 1.4 b	54.8 ± 2.7 a	55.1 ± 1.8 a
10th week	54.0 ± 1.8 a	64.2 ± 1.2 b	55.6 ± 1.3 a	54.8± 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 $CaNa_2EDTA$ macroparticles or nanoparticles.**

Table (4): Mean values and ± S.E of the monocytes percentage in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50mg/kg/day $CaNa_2EDTA$ 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	2.9 ± 0.4 a	3.5 ± 0.6 a		
4th week	3.8 ± 0.6 a	4.3 ± 0.5 a		
6th week*	2.5± 0.3 a	3.5 ± 0.4 a	Cadmium & $CaNa_2EDTA$ Macroparticles	Cadmium & $CaNa_2EDTA$ Nanoparticles
8th week	1.8 ± 0.2 a	2.1 ± 0.2 a	2.5 ± 0.3 ac	2.9 ± 0.3 c
10th week	1.8 ± 0.2 a	2.0 ± 0.3 a	2.3 ± 0.2 a	2.4± 0.2 a

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

***Start of the treatment with $CaNa_2EDTA$ macroparticles or nanoparticles.**

Neutrophils Phagocytic Activity Percentage

The neutrophils of both control rats and those intoxicated by cadmium and treated with $CaNa_2EDTA$ nanoparticles showed significantly high phagocytic activities compared with that of the cadmium intoxicated and not treated rats, which showed a significant decrease in their neutrophils' phagocytic activity. Treatment with $CaNa_2EDTA$ macroparticles slightly restored the decrease in neutrophils' phagocytic activity exerted by cadmium with insignificant differences between them (Table 5).

Table (5): Mean values ± S.E of the neutrophils phagocytic activity percentage in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50 mg/kg/day $CaNa_2EDTA$ 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	74.5 ± 2.3 a	61.5 ± 1.0 b		
4th week	74 ± 2.3 a	56 ± 1.6 b		
6th week*	68.8 ± 2.3 a	55.8 ± 2.0 b	Cadmium & $CaNa_2EDTA$ Macroparticles	Cadmium & $CaNa_2EDTA$ Nanoparticles
8th week	64.4 ± 0.8 a	51.7 ± 1.7 b	57.7 ± 4.0 abc	68.5 ± 1.5 a
10th week	72.5 ± 2.5 a	57.5 ± 2.5 b	65 ± 0.6 abc	68 ± 3.6 ac

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

***Start of the treatment with $CaNa_2EDTA$ macroparticles or nanoparticles.**

Electrophoresis Pattern of Serum Protein:

The SDS-PAGE electrophoresis pattern of serum protein fractions at the end of the experiment in control and all the experimental groups are presented in (Table 6). Albumin level showed a significant difference among the control and the experimental groups. The $CaNa_2EDTA$ nanoparticles group showed a closer albumin level to the control group than the $CaNa_2EDTA$ macroparticles group. The albumin level of the cadmium group was very low when compared to the control and the other experimental groups. B-globulin level of the

cadmium group was significantly higher than that of the control group, while α and ɣ-globulins levels were significantly lower than that of the control group. In contrast to these findings, $CaNa_2EDTA$ macro and nanoparticles groups effectively reduced the increase in β-globulin level and increased α and ɣ-globulins levels.

Table (6): Mean values ± S.E of the serum protein fractions (gm/dL) at the end of the experiment in control and experimental rats received drinking water contain 30ppm cadmium and intraperitoneally injected with 50mg/kg/day $CaNa_2EDTA$ macroparticles or nanoparticles at the end of the 6^{th} till the 10^{th} week of the experiment for 4 courses (4 days each) with 3 days interval between the courses.

Groups / Proteins	Control	Cadmium	$CaNa_2EDTA$ Macroparticles	$CaNa_2EDTA$ Nanoparticles
Albumin	**6.4 ± 0.02 a**	**5.3 ± 0.04 b**	**6.1 ± 0.01 c**	**6.2 ± 0.06 d**
α-Globulin	**1.16 ± 0.01 a**	**1.11 ± 0.01 b**	**1.18 ± 0.01 ac**	**1.19 ± 0.01 c**
β-Globulin	**1.1 ± 0.02 a**	**1.7 ± 0.08 b**	**1.3 ± 0.02 ac**	**1.0 ± 0.08 a**
ɣ-Globulin	**1.3 ± 0.02 a**	**0.7 ± 0.02 b**	**1.0 ± 0.02 c**	**1.1 ± 0.03 d**

Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n = 8).

DISCUSSION

Cadmium is one of the most important environmental and occupational metallic toxicants and is widely dispersed in the environment. High-level exposure to this toxic heavy metal is usually the result of environmental contamination from human activities. Exposure to Cd can cause both acute and chronic tissue injury and can damage various organs in human beings and experimental animals **(Sarkar *et al.*, 1995).**

In the current study, cellular immune response as estimated by; the total and differential leucocytic counts and phagocytic activities of neutrophils was severely reduced in cadmium intoxicated rats when they compared with the treated groups with $CaNa_2EDTA$ macro or nanoparticles especially the nanoparticles form except the neutrophils and monocytes percentages which showed a marked elevation in cadmium intoxicated rats. These findings

suggested that nanoparticles of $CaNa_2EDTA$ alleviated these effects by chelating cadmium and consequently preventing it from inducing its toxic effects on the immune system.

Leucogram of cadmium-intoxicated rats showed leucopenia with lymphopenia.These changes may be attributed to the direct toxic effect of heavy metals on blood cells or indirectly on the hemopoietic organs. Neutrophils and monocytes percentages markedly increased in cadmium-intoxicated rats that could be explained by the inflammatory response elicited by cadmium. **Tinkov *et al.,* (2018)** recorded that Cd exposure induces inflammatory response and cell damage including disruption of tight junctions in intestinal wall, ultimately leading to increased gut permeability. Together with increased lipopolysaccharide (LPS) production, impaired barrier function causes endotoxinemia and systemic inflammation. **McMarry *et al*., (1995); Karmakar *et al.,* (2000) and Randa *et al.,* (2012),** reported similar results**.** According to the present study, humoral immune response as estimated by the measurement of electrophoresis pattern of serum proteins (albumin, alpha, beta and gamma globulins) reported a significant inhibition in cadmium intoxicated rats except levels of beta globulins which showed a significant increase which could be explained by the effect of cadmium on hematopoeisis and subsequently the resulting anemia. The Anemia could be in association with marked suppression of erythropoietin **(Horiguchi *et al.,* 1994)** or hemolysis **(Horiguchi *et al.,* 2011). Randa *et al.,* (2012),** reported similar results**.** Liver is known to play an essential role in the synthesis of plasma protein and some globulins. In addition, kupffer cells of the liver are known for their antigenicity as they phagocytose antigens. The toxicity of cadmium may involve a reaction of reactive oxygen species (ROS) as described by **Manca *et al*., (1991)**. Activated neutrophils produce ROS during inflammatory reactions and if neutrophils accumulate, tissue will be exposed to large quantities of potentially injurious neutrophil content **(Rollet- Labella *et al*., 1998).** A plausible mechanism may involve cadmium concentration in hepatic tissue following exposure. Cadmium concentration may exceed the intracellular metallothionein and glutathione concentration enabling cadmium to interact with the cellular organelles, which could disrupt biochemical processes. Subchronic administration of cadmium to mice resulted in a hepatic injury due to disruption of DNA replication, RNA synthesis and mitochondrial metabolism **(Karmakar *et al*., 2000)**. **Luckey *et al*., (1975)** recorded that cadmium induced impaired renal tubular reabsorption of serum protein, which contribute in part to hypoproteinemia.

Gamma globulins were severely reduced in cadmium-intoxicated rats. These results came in accordance with the recorded data of **Randa *et al.*, (2012).** The results also agree with **Ohsawa *et al.*, (1988)** who reported that when mice were primed with sheep red blood cells after exposure to $CdCl_2$, a significant suppression of the antibody forming response was observed in animals fed 300 ppm $CdCl_2$, but not in those fed 3 ppm of the same salt. **Daum *et al*., (1993)** mentioned that $CdCl_2$ exerted an early inhibitory effect on B- cell activation. This was attributed to the inhibition of RNA, DNA and antibody synthesis. **Karmakar *et al*., (2000)** reported highly significant depleted value of lymphocytes in the 21st day post exposure of mice to subcutaneous injection with cadmium chloride (2.5 mg/ kg body weight). By measuring the hemagglutination titer and delayed type hypersensitivity response, the results of **Lall and Dan, (1999)** indicated the involvement of adrenal hormones in cadmium-induced immunosuppression suggesting that cadmium activates the corticosteroid associated immune-regulatory circuit.

Dong *et al*. (2002) mentioned that cadmium stimulated and increased beta adrenoreceptor density of rat splenic cell membrane. The immunotoxicity of cadmium showed that, the proliferation of T- lympocytes was inhibited and the subsets of T-cells (CD4 +, CD8 +, CD4+ /CD8+) were changed.

In the groups, which intoxicated with cadmium and treated with $CaNa_2EDTA$ macro or nanoparticles, humoral immune response was improved. More improvement was recorded in the nanoparticles treated group as revealed by the elevation in the total serum protein and its fractions (albumin, and alpha and gamma globulins) due to the protection of the liver from the toxic effects of cadmium by their cadmium chelating power. The beta globulins level was reduced and became more close to the control group.

The protective role of $CaNa_2EDTA$ nanoparticles seems to be due to reduction in size and difference in shape of the nanoformulation, which is evenly spherical because of precipitation process. This provides an optimization to their use as a chelating agent for cadmium toxicity and thus minimizing its toxic effects. While, macronized forms is irregular shape resulting from machining or grinding processes **(Kumar *et al.*, 2011).**

In conclusion, this study recommends 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 immunotoxic effects of cadmium in rats.

REFERENCES

Abernethy, D. R.; De-Stefano, A. J.; Cecil, T. L.; Zaidi, K. and Williams, R. L. (2010): Metal impurities in food and drugs. Pharm. Res., 27 (5): 750 -755.

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.

Chatterjee, S.; Kundu, A.; Sengupta, S. and Bhattacharyya, A. (2009): Divergence to apoptosis from ROS induced cell cycle arrest: effect of cadmium. Mutat. Res., 663 (1-2): 22 31.

Daum, J. R.;Shepherd, D. M. and Noelle, R. J. (1993): Immunotoxicology of cadmium and mercury on B-lymphocytes-I. Effects on lymphocyte function. Int. J. Immunopharmacol.,15 (3): 383-394.

Donald,B.C.;Edward,A.andAshwood,A.D.(1996): Tiezts-Fundermental Chemistry.416.p 145.

Dong, S.; Li, D. and Yu, G. (2002): Effect of cadmium on the beta- adrenoreceptor of splenic cell of rats and its immunotoxicity. Wei sheng yan jiu, 28 (4): 203 - 205.

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.

Fortier, M.; Omara, F.; Bernier, J.; Brousseau, P. and Fournier, M. (2008): Effects of physiological concentrations of heavy metals both individually and in mixtures on the viability and function of peripheral blood human leukocytes in vitro, J. Toxicol. Environ. Health A., 71(19):1327-1337.

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.

Giffored, R. H.; Malawista, S.E. (1970): A simple rapid Micro Method for detecting chronic granulomatous for childhood. J.lab. Clin. Med. 75 (3): 511-518.

Hanson, M. L.; Brundage, K. M.; Schafer, R.; Tou, J. C. and Barnett, J. B. (2010): Prenatal cadmium exposure dysregulates sonic hedgehog and Wnt/β-catenin signaling in the thymus resulting in altered thymocyte development. Toxicol. Appl. Pharmacol., 242(2): 136-145.

Hanson, M. L.; Holaskova, I. and Elliott, M. (2012): Prenatal cadmium exposure alters postnatal cell development and function, Toxicol. Appl. Pharmacol., 261(2): 196-203.

Horiguchi, H.; Oguma, E. and Kayama, F.(2011): Cadmium induces anemia through interdependent progress of hemolysis, body iron accumulation, and insufficient erythropoietin production in rats. Toxicol. Sci., 122 (1): 198 -210.

Horiguchi, H.; Teranishi, H.; Niiya K.; Aoshima, K.; Katoh, T.; Sakuragawa, N. and Kasuya, M. (1994): Hypoproduction of erythropoietin contributes to anemia in chronic cadmium intoxication: clinical study on Itai-itai disease in Japan. Arch. Toxicol., 68(10): 632 - 636.

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.

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

Karmakar, R.; Bhattacharya, R. and Chatterjee, M. (2000): Biochemical, haematological and histopathological study in relation to time-related cadmium- induced hepatotxicity in mice. BioMetals, 13: 231-239.

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.

Lall, S. B. and Dan, G. (1999): Role of corticosteroids in cadmium induced immunotoxicity. Drug Chem.Toxicol, 22 (2): 401 - 409.

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

Luckey, T.; Venugopal, B. and Hutcheson, D. (1975): Heavy metal toxicity. 1st ed Geory Thieme publishers, Stuttgart, Academic Press, Inc, New York, San Francisco, London.

Manca, D.; Ricard, A. C.; Trottier, B. and Chevalier, G. (1991): Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicol, 67(3): 303-323.

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.

McMurry, S.; Lochmiller, L.; Chandra, S. and Qualls, R. (1995): Sensitivity of selected immunological hematological and reproductive parameters in the cotton rat (Sigmodon hispidus) to subchronic lead exposure. J. Wildl. Dis., 31 (2): 193-204.

National Toxicology Program (2004): Report on Carcinogens, Eleventh edition. Carcinogen profiles, 2004. Research Triangle Park, North Carolina: US Department of Health and Human Services, Public Health Service, National Toxicology Program.

Navas-Acien, A.; Selvin,E. and Sharrett, A. R.; Calderon-Aranda, E.; Silbergeld, E. and Guallar, E. (2004): Lead, cadmium, smoking, and increased risk of peripheral arterial disease. Circulation, 109 (25): 3196 -3201.

Ohsawa, M. (2009): Heavy metal-induced immunotoxicity and its mechanisms. Yakugaku Zasshi., 129(3): 305 - 319.

Ohsawa, M.; Takahashi, K. and Otsuka, F. (1988): Induction of anti- nuclear antibodies in mice orally exposed to cadmium at low concentrations. Clin. Exp. Immunol., 73 (1): 98-102.

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.

Rollet-Labella, E.; Grange, M. J.; Elbim, C.; Marouetty, C.; Gougerot- Picadalo, M. A. and Pasquier, C. (1998): Hydroxyl radical as a intracellular mediator of polymorphonuclear neutrophil apoptosis. Free Radic. Biol. Med., 24 (4): 563- 572.

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.

Sarkar, S.; Yadav, P.; Trivedi, R.; Bansal, A. K. and Bhatnagar, D. (1995): Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J. Trace Elem. Med. Biol., 9 (3): 144 -149.

Schalm, O. W.; Jain, N.C and Carrol, E. J. (1975): Veterinary Haematology, 3rd Edn, Lea and Febiger, Philadelphia, pp. 197-199.

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.

U. S. Geological Survey (2012): Mineral Commodity Summaries, U.S. Geological Survey, Rolla, Mo, USA.

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.

Waisberg, M.; Joseph, P.; Hale, B. and Beyersmann, D. (2003): Molecular and cellular mechanisms of cadmium carcinogenicity. Toxicology, 192 (2-3): 95-117.

Wang, H. and Hurley, W. L. (1998): Identification of lactofrien complexes in bovine mammary secretion during mammary gland involution. J. Dairy Sci. 81: 1896 -1903.