

Research Article



Evaluation of The Protective Effect of Zinc Oxide / Ascorbyl Palmitate Nano-Composite on Cadmium - Induced Hepatotoxicity and Nephrotoxicity in Rats

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ABSTRACT

Cadmium (Cd) is one of the most toxic heavy metals. This metal is a serious environmental and occupational contaminant and may represent a serious health hazard to humans and other animals. Chronic exposure to Cd causes hepatotoxicity and nephrotoxicity. The present study was designed to investigate the antioxidant effect of ZnO/Ascorbyl palmitate nano-composite on cadmium chloride (CdCl₂) induced liver and kidney damage in Sprague-Dawley rats. Subcutaneous administration of Cd (2mg/kg b.w.) 5 days a week as CdCl₂ for 30 days resulted in a significant increase in the levels of serum Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Urea and Creatinine and a significant decrease in the levels of liver and kidney antioxidants namely, Reduced glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) along with significant elevation in the level of Lipid peroxidation (TBARS) when compared with the control group. Oral administration of ZnO/Ascorbyl palmitate nano-composite (25 mg/kg b.w.) 5 days a week for 30 days with CdCl₂ induced improvement in all examined parameters. The protective effect of ZnO/Ascorbyl palmitate nano-composite in respect to biochemical changes were also confirmed by histopathological study in the liver and kidney sections. Our results suggest that ZnO/Ascorbyl palmitate nano-composite may attenuate cadmium-induced oxidative damage in the liver and kidney of rats.

Keywords: Cadmium chloride, Hepatotoxicity, Histopathology, Nephrotoxicity, Antioxidants, Oxidative stress.

INTRODUCTION

Exposure to heavy metals has become an increasingly recognized source of illness worldwide.¹ Most, if not all, metals are toxic, even those known to be essential.² Cd is a very toxic heavy metal and an important environmental pollutant, which is present in the soil, water, air, food and in cigarette smoke.³ Cd can cause human health problems through occupational and environmental exposure. It affects cell proliferation, differentiation, apoptosis and other cellular activities. Cd causes poisoning in various tissues of humans and animals.⁴ Prolonged exposure to Cd results in injury to the liver, lungs, kidney and testes.⁵ Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage.⁶

Cd acts as a catalyst in forming reactive oxygen species (ROS). It increases lipid peroxidation, in addition it depletes antioxidants, glutathione and protein-bound sulfhydryl groups. It also promotes the production of inflammatory cytokines.⁷ Many studies suggested that generation of ROS and its interference with cellular antioxidant system is one of the major mechanisms by which toxic effect of Cd is mediated.⁸ As oxidative stress is one of the important mechanisms of cadmium-induced damages, it can be expected that the administration of some antioxidants should be an important therapeutic approach.^{9,10}

Nanostructure materials, primarily defined by unique properties and determined interaction with other disciplines, can interact with biological systems at basic molecular levels with high specificity. Making use of this molecular feature, nanodevices can stimulate and interact with objective cells in certain ways to induce and maximize desired physiological responses.^{11,12} They've been recognized for their potential biological utility including biological science and nanomedicine.^{13,14} Zinc oxide (ZnO) nanopowders are available as powders and dispersions. These nanoparticles exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties, used in diverse applications ranging from paints and cosmetics to biomedicine and food.^{15,16} Some studies have indicated that nano ZnO affected functions of different cells or tissues^{17,18}, biocompatibility¹⁹ and neural tissue engineering.^{11,12}

Ascorbyl palmitate is a fat-soluble form of vitamin C, known as vitamin C ester which is better absorbed than ascorbic acid, the water-soluble form. It offers all the benefits of ascorbic acid, plus it won't flush out of the body as quickly as ascorbic acid, and it is able to be stored in cell membranes until the body needs it. Ascorbyl palmitate is an amphipathic molecule, which means one end is water-soluble and the other end is fat-soluble. This dual solubility allows it to permeate the extra-cellular aqueous environment of the cell and the interior cellular environment as well.²⁰ Ascorbyl palmitate has antioxidant properties, it is a powerful free radical scavenger²¹, when it is incorporated into the cell membranes of human red



blood cells, Ascorbyl palmitate protects them from oxidative damage and also helps protect vitamin E (a fat-soluble antioxidant) from oxidation by free radicals.²⁰

Therefore, the aim of this study was to evaluate the antioxidant effect of ZnO/Ascorbyl palmitate nano-composite against CdCl₂- induced liver and kidney damage in rats.

MATERIALS AND METHODS

Chemicals: CdCl₂ was purchased from ICN pharmaceutical company (USA). ZnO nanoparticles were purchased from Merck chemicals (Germany). All other chemicals and solvents used in this study were of highest purity and analytical grade, and purchased from Sigma-Aldrich chemie (Deisenhofer, Germany).

Preparation of ZnO/Ascorbyl palmitate nano- composite

The nano-composite was prepared by coating ZnO nanoparticle (10-54nm) with Ascorbyl palmitate dissolved in dimethylformamide (DMF) at 60°C. The necessary amount 1 gm ZnO nanoparticle (10-54nm) surface area 15-25 m²/g was gradually added to 4 gm Ascorbyl palmitate in DMF solution under stirring and heating resulting in a suspension with homogeneous appearance. The suspension was maintained, stirred, and heated until almost complete solvent evaporation. The material presenting a creamy consistency was washed several times with distilled water, under vigorous stirring to remove residues and remaining organic solvent. Next, the composite is dried at 70°C for 24 hours to give ZnO/Ascorbyl palmitate nano-composite. The morphology and the crystallizing of ZnO/Ascorbyl palmitate nano-composite examined by transmission electron microscope (TEM) JOEL JEM 1230 (made in Japan), working at 120 Kev.

Cytotoxicity determination

Median lethal dose (LD50) of ZnO/Ascorbyl palmitate nano-composite was calculated using the method of Prieur et al.²² and Ghosh.²³ In order to determine a non toxic dose of ZnO/Ascorbyl palmitate nano-composite, the rats were subjected to series of different concentrations of nano-composite from 10 mg to 40 mg/kg body weight (b.w.) suspended in saline, given orally daily by gavage for one month. These concentrations were given to 10 rats for each concentration. It was showed that 25 mg/kg (b.w.) was safe and non toxic to experimental rats.

Animals

Male Sprague-Dawley rats each weighing about 150–180 g, were obtained from the animal house of the National Research Center, Egypt. Animals were housed in well-aerated polycarbonate cages under standard laboratory conditions (30±2°C; light: dark=1:1 cycle; humidity (55 ± 10%). The animals were fed with a commercial pellet diet and had access to water *ad libitum* and were acclimatized for a week before commencement of all

experiments. Ethical approval for the handling of experimental animals was obtained from the institutional animal ethics committee of the National Research Centre., Egypt.

Experimental Design

They were randomly divided into five groups of 6 rats each as following:

Group 1: Control rats treated subcutaneously (sc) with 0.9% NaCl

Group 2 (nano-composite group): Rats were received ZnO/Ascorbyl palmitate nano-composite (25 mg/kg b.w. suspended in saline) orally 5 days a week using intragastric tube for 30 days.

Group 3 (Cd group): Rats were injected subcutaneously with Cd as CdCl₂ (2mg/kg b.w. dissolved in saline)²⁴ 5 days a week for 30 days.

Group 4 (nano-composite pre-treated group): Rats were received ZnO/Ascorbyl palmitate nano-composite as group 2 started one week before the first dose of CdCl₂ treatment.

Group 5 (nano-composite post-treated group): Rats were post-treated with ZnO/Ascorbyl palmitate nano-composite as group 2 after one week from the administration of CdCl₂ as group 3 and continued until the end of the experiment.

Blood collection

At the end of experiment, animals were anesthetized and blood samples were collected from ocular vascular bed using capillary tubes. Blood samples were collected into dry clean tubes then centrifuged at 3000 rpm for 15 min. to separate serum and stored at -20°C.

Tissue samples

Animals were killed by cervical decapitation. Liver and kidney were rapidly removed, washed in ice-cold saline, weighed and blotted dry. A known weight of these tissues was homogenized (10% w/v) in ice cold phosphate buffer (0.1 M, pH 7.4) using Omni tissue master homogenizer. The homogenate was centrifuged at 3000 rpm at 4 °C for 10 minutes and the resulting supernatant was stored at -70 °C for biochemical analysis.

Biochemical analysis

Serum samples collected from different groups were analyzed for AST, ALT, ALP, GGT, Urea and Creatinine using kits supplied by Quimica Clinica Aplicada S.A (Spain) according to the manufacturer's instructions. Liver and kidneys tissue homogenates were used for the estimation of the content of GSH according the methods of Beutler et al.²⁵, GPx, SOD, CAT activities were determined according to Necheles et al.²⁶, Marklund and Marklund²⁷, and Sinha²⁸ respectively. The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Conrad et al.²⁹.



Histopathological Studies

Immediately after sacrifice, Small pieces of liver and kidney tissues were fixed in 10% formalin solution, dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced section (5 μm thickness), and stained with haematoxylin-eosin dye and then observed under microscope.³⁰

Statistical analysis

All studied data were evaluated with SPSS/19 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test which was used to identify differences between group means. *P* values of < 0.05 were considered as the minimum level of significant. The data were expressed as mean \pm S.E with six animals in each group.

RESULTS

Characterization of ZnO/Ascorbyl palmitate nano-composite

On examining the prepared powder of nano-composite as seen in figure 1 (A,B) by TEM micrograph. Figure 1a shows that the prepared sample composed of self assembling of very fine particles forming a uniform nanostructure with particle size range from 10nm-54nm, where the inset figure in figure 1A showed the morphology of pure nanoparticle ZnO. While, the corresponding selected area electron diffraction (SAED) in figure 1B revealed a crystalline structure with preferred orientation, this may be due to presence of nano crystal of ZnO.

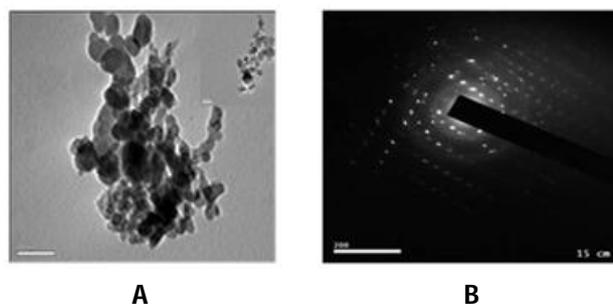


Figure 1: (A) TEM image of ZnO/Ascorbyl palmitate nano-composite showing particles size 10-54 nm, (B) SAED revealed a crystalline structure of ZnO/Ascorbyl palmitate nano-composite.

Biochemical results

The body weight of rats exposed to Cd revealed significant decreased values as compared to control group, while the administration of pretreated nano-composite resulted in a significant increase in body weight when compared to Cd group. Liver and kidney weights in Cd and post treatment of nano-composite groups showed a significant elevation as compared to control group, while the liver and kidney weights were reduced in pre-treated nano-composite comparing to Cd group. Also the relative weights of liver and kidney revealed a significant lower in pre-treatment than post-treatment of nano-composite as compared to Cd group (table 1).

Table 1: Effect of ZnO/Ascorbyl palmitate nano-composite on body, liver and kidney weights in CdCl₂ treated rats

| GROUP | INITIAL BODY WEIGHT | FINAL BODY WEIGHT | LIVER WEIGHT | RELATIVE LIVER WEIGHT | KIDNEY WEIGHT | RELATIVE KIDNEY WEIGHT |
|-------------------------------|--------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| CONTROL | 236.83 \pm 2.27 | 261.16 \pm 2.15 | 7.64 \pm 0.25 | 2.92 \pm 0.10 | 1.70 \pm 0.02 | 0.65 \pm 0.01 |
| NANO-COMPOSITE | 241.03 \pm 1.91 | 261.92 \pm 3.49 | 7.84 \pm 0.17 | 2.99 \pm 0.08 | 1.73 \pm 0.04 | 0.66 \pm 0.02 |
| CD | 245.50 \pm 1.66 ^A | 211.16 \pm 2.95 ^A | 10.43 \pm 0.16 ^A | 4.94 \pm 0.13 ^A | 2.17 \pm 0.05 ^A | 1.02 \pm 0.03 ^A |
| NANO-COMPOSITE PRE-TREATMENT | 244.62 \pm 1.83 ^A | 255.72 \pm 1.56 ^B | 8.27 \pm 0.24 ^B | 3.23 \pm 0.10 ^B | 1.79 \pm 0.05 ^B | 0.69 \pm 0.02 ^B |
| NANO-COMPOSITE POST-TREATMENT | 246.04 \pm 1.21 ^A | 236.96 \pm 2.33 ^{AB} | 9.69 \pm 0.26 ^{AB} | 4.09 \pm 0.12 ^{AB} | 1.98 \pm 0.05 ^{AB} | 0.83 \pm 0.02 ^{AB} |

Values are expressed as mean \pm SE (n=6), a: the Cd-chloride group was compared to the control group. b: treated group was compared to Cd-chloride group, significant at P<0.05.

Table 2: Effect of ZnO/Ascorbyl palmitate nano-composite on serum liver functions in CdCl₂ treated rats.

| GROUP | GOT (U/ML) | GPT (U/ML) | GGT (U/L) | ALP (U/L) |
|-------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| CONTROL | 21.33 \pm 1.02 | 23.50 \pm 1.28 | 4.20 \pm 0.63 | 39.01 \pm 1.23 |
| NANO-COMPOSITE | 22.50 \pm 1.94 | 24.16 \pm 1.37 | 4.53 \pm 0.46 | 41.27 \pm 1.81 |
| CD | 101.66 \pm 11.94 ^A | 70.83 \pm 6.05 ^A | 14.67 \pm 0.85 ^A | 133.13 \pm 10.53 ^A |
| NANO-COMPOSITE PRE-TREATMENT | 44.83 \pm 2.44 ^{A,B} | 41.16 \pm 1.40 ^{A,B} | 7.99 \pm 0.61 ^{A,B} | 58.52 \pm 3.06 ^{A,B} |
| NANO-COMPOSITE POST-TREATMENT | 54.83 \pm 3.85 ^{A,B} | 48.66 \pm 2.96 ^{A,B} | 11.07 \pm 0.76 ^{A,B} | 72.15 \pm 3.87 ^{A,B} |

Values are expressed as mean \pm SE (n=6), a: the Cd-chloride group was compared to the control group. b: treated group was compared to Cd-chloride group, significant at P<0.05.

Liver enzymes represent the levels of ALT, AST, ALP, and GGT in the serum of control and all experimental rat groups are shown in Table 2. The results showed that Cd Cl₂ injection induced a significant increase in the activities of these enzymes in the Cd group compared to those values in control group. There was no significant change in the levels of these enzymes in ZnO/Ascorbyl palmitate nano-composite group compared to normal rats. The activities of ALT, AST, ALP, and GGT enzymes were significantly decreased in those rats pre-treated with nano-composite, and those post-treated with nano-composite compared to the Cd group. However, the ameliorative effect of pre-treatment with nano-composite was more pronounced than that of post-treatment with nano-composite.

In the present study, CdCl₂ treatment caused nephrotoxicity as evidenced by significant elevation in serum urea and creatinine in the Cd group when compared with the control group (Table 3). However, levels of serum urea and creatinine were significantly decreased in nano-composite pre-treated group and nano-composite post-treated group compared with the Cd group. The ameliorative effect of pre-treatment with nano-composite on the levels of serum urea and creatinine was more prominent than that of post-treatment with nano-composite (Table 3). The administration of nano-composite alone to rats did not affect the levels of all these parameters as compared to the control group.

Table 3: Effect of ZnO/Ascorbyl palmitate nano-composite on serum kidney functions in CdCl₂ treated rats

| GROUP | UREA (MG/DL) | CREATININE (MG/DL) |
|-------------------------------|-----------------------------|----------------------------|
| CONTROL | 27.92 ± 1.67 | 0.56 ± 0.05 |
| NANO-COMPOSITE | 28.74 ± 1.21 | 0.67 ± 0.04 |
| Cd | 94.12 ± 4.22 ^A | 1.96 ± 0.18 ^A |
| NANO-COMPOSITE PRE-TREATMENT | 38.12 ± 1.07 ^{A,B} | 1.06 ± 0.04 ^{A,B} |
| NANO-COMPOSITE POST-TREATMENT | 45.75 ± 2.11 ^{A,B} | 1.28 ± 0.09 ^{A,B} |

Values are expressed as mean ± SE (n=6), a: the Cd-chloride group was compared to the control group. b: treated group was compared to Cd-chloride group, significant at P<0.05.

Tables 4 and 5 show the activities of GPx, SOD, and CAT as well as TBARS and GSH levels in liver and kidney of control and experimental groups of rats. In the Cd group, the activities of liver and kidney GPx, SOD, and CAT were significantly reduced as a result of CdCl₂ administration when compared with the control group. Comparing to the Cd group, the activities of these enzymes were significantly increased in pre-treated group with nano-composite. However, the pre-treatment with nano-composite showed more improvement in these activities than post-treatment as there were insignificant values between their levels in nano-composite post-treated group and Cd group (Table 4).

Table 4: Effect of ZnO/Ascorbyl palmitate nano-composite on liver antioxidant enzymes and lipid peroxidation in CdCl₂ treated rats

| GROUP | GSH (µg/G TISSUE) | GPx (µMOL/MIN/G TISSUE) | SOD (µg/G TISSUE) | CAT (µMOL/MIN/G TISSUE) | TBARS (µMOL/G TISSUE) |
|-------------------------------|----------------------------|--------------------------|-----------------------------|-----------------------------|-----------------------------|
| CONTROL | 189.66±9.08 | 8.31±0.25 | 358.23±37.96 | 461.66±22.88 | 151.35±7.40 |
| NANO-COMPOSITE | 150.16±7.58 | 8.42±0.19 | 321.44±22.49 | 329.50±12.91 | 185.16±6.78 |
| Cd | 58.16±1.66 ^A | 5.18±0.14 ^A | 133.93±5.07 ^A | 250.66±7.71 ^A | 426.62±49.09 ^A |
| NANO-COMPOSITE PRE-TREATMENT | 100.50±6.16 ^{A,B} | 5.97±0.21 ^{A,B} | 264.51±19.73 ^{A,B} | 310.83±13.93 ^{A,B} | 193.89±8.51 ^B |
| NANO-COMPOSITE POST-TREATMENT | 88.83±3.36 ^{A,B} | 5.34±0.26 ^A | 160.58±10.98 ^A | 289.50±6.64 ^A | 240.07±14.29 ^{A,B} |

Values are expressed as mean ± SE (n=6), a: the Cd-chloride group was compared to the control group. b: treated group was compared to Cd-chloride group, significant at P<0.05.

Table 5: Effect of ZnO/Ascorbyl palmitate nano-composite on kidney antioxidant enzymes and lipid peroxidation in CdCl₂ treated rats

| GROUP | GSH (µg/G TISSUE) | GPx (µMOL/MIN/G TISSUE) | SOD (µg/G TISSUE) | CAT (µMOL/MIN/G TISSUE) | TBARS (µMOL/G TISSUE) |
|-------------------------------|----------------------------|--------------------------|-----------------------------|-----------------------------|----------------------------|
| CONTROL | 147.66±7.44 | 8.52±0.23 | 431.09±23.77 | 351.83±11.51 | 155.33±9.32 |
| NANO-COMPOSITE | 138.00±8.71 | 8.13±0.15 | 399.13±32.11 | 302.66±8.63 | 190.40±6.35 |
| Cd | 62.66±4.44 ^A | 5.14±0.19 ^A | 135.72±10.16 ^A | 258.00±4.49 ^A | 262.94±19.11 ^A |
| NANO-COMPOSITE PRE-TREATMENT | 105.33±7.09 ^{A,B} | 5.92±0.08 ^{A,B} | 315.42±12.60 ^{A,B} | 306.33±10.77 ^{A,B} | 180.19±7.91 ^B |
| NANO-COMPOSITE POST-TREATMENT | 99.16±4.96 ^{A,B} | 5.44±0.13 ^A | 199.76±14.31 ^{A,B} | 274.00±4.65 ^A | 206.89±9.25 ^{A,B} |

Values are expressed as mean ± SE (n=6), a: the Cd-chloride group was compared to the control group. b: treated group was compared to Cd-chloride group, significant at P<0.05.

Cd group showed a significant decrease in the level of GSH and a significant increase in the level of TBARS compared to the control group. Cd group pre-treated and post-treated with nano-composite were found to significantly increase GSH level and significantly decrease TBARS level compared with the Cd group (Tables 4,5). However, there was no significant difference between levels of TBARS in Cd group pre-treated with nano-composite and their levels in control group. The levels of liver and kidney antioxidant were non significantly different in rats administered with nano-composite alone when compared to the control group (Tables 4,5).

Histological investigation

In the present histopathological study, the liver sections of normal rats and rats treated with nano-composite revealed preserved hepatic lobular architecture and normal hepatocytes with rounded vesicular nuclei figure 2 (A,B). Treatment of rats with CdCl₂ caused focal portal infiltration by chronic nonspecific inflammatory cells rich in lymphocytes, and congested sinusoids. The hepatocytes showed focal marked eosinophilic cytoplasm with nuclear granular degenerative changes, condensed nuclear chromatin and congested dilated portal vessels figure 2(C) (1-2). The pre-treated of nano-composite showed reduced infiltration by inflammatory cells, minimal sinusoidal congestion and unremarkable individual hepatocellular changes figure 2(D). The post-treatment with nano-composite revealed reduced focal portal infiltration by chronic nonspecific inflammatory cells rich in lymphocytes, and congested sinusoids. The hepatocytes showed minimal focal eosinophilic cytoplasm figure 2(E).

Histological study of the kidneys of the control rats and rats treated with nano-composite revealed preserved renal architecture, normal nephron and renal tubules were free of any pathological changes figure 3(A,B). The treatment of rats with CdCl₂ induced minimal marked alterations in renal tissues when compared to control rats. These changes were in the form of focal cytoplasmic tubular epithelial degeneration, and interstitial edema figure 3(C). The pre and post-treatment with nano-composite improved the kidney histology, where exhibited minimal interstitial edema, normal nephron, and normal epithelial lining the tubules figure 3 (D,E).

DISCUSSION

Cd is one of the most dangerous occupational and environmental toxins. Products of vegetable origin are the main carrier of cadmium compounds in food.³¹ Many studies suggested that generation of reactive oxygen species (ROS) and its interference with cellular antioxidant system is one of the major mechanisms by which toxic effect of Cd is mediated.⁷ Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein and DNA damage.³² This possibly leads to the depletion of the

body's endogenous antioxidants which serve as a premier source of protection against free radicals.³³

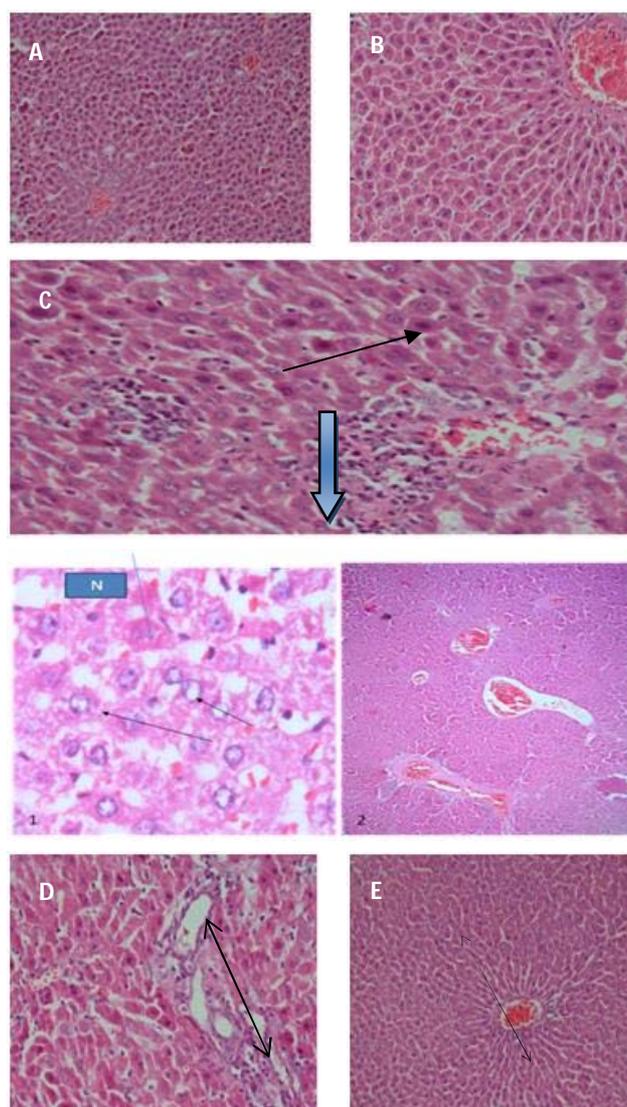


Figure 2: Photomicrograph of (A) normal control liver tissue with preserved hepatic lobular architecture, normal hepatocytes and central vein free of any pathological changes (Hematoxylin & Eosin stain; x100), (B) liver tissue of rat treated with nano-composite showing no pathological changes (Hematoxylin & Eosin stain; x200), (C) (1-2) liver tissue of rat treated with Cd showing focal portal infiltration by chronic nonspecific inflammatory cells rich in lymphocytes, and congested sinusoids (thick arrow). The hepatocytes showed focal marked eosinophilic cytoplasm with nuclear (N) granular degenerative changes and condensed nuclear chromatin (thin arrow). Congested dilated portal vessels (V) (Hematoxylin & Eosin stain; x200), (D) liver tissue of rat pre-treated with nano-composite showing reduced infiltration by inflammatory cells (thin arrow), minimal sinusoidal congestion and unremarkable individual hepatocellular changes (Hematoxylin and Eosin; x200) and (E) liver tissue of rat post-treated with nano-composite showing reduced focal portal infiltration by chronic nonspecific inflammatory cells rich in lymphocytes, and

congested sinusoids. The hepatocytes showed minimal focal eosinophilic cytoplasm (Hematoxylin & Eosin stain; x100).

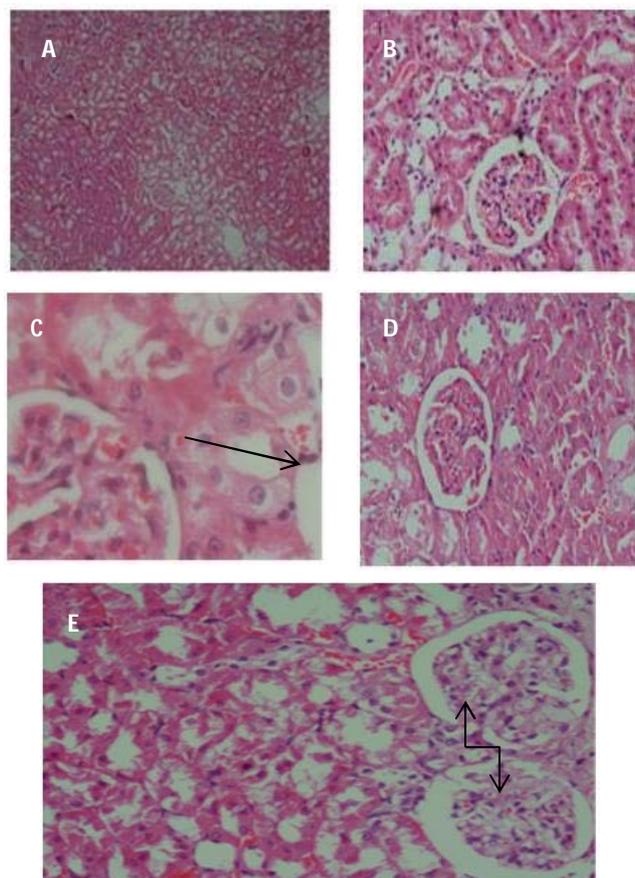


Figure 3: Photomicrograph of (A) normal control kidney tissue showing preserved renal architecture, normal nephron and renal tubules are free of any pathological changes (Hematoxylin & Eosin stain; x100), (B) renal tissue of rat treated with nano-composite showing no pathological changes (Hematoxylin & Eosin stain; x200), (C) renal tissue of rat treated with Cd showing normal nephron, focal cytoplasmic tubular epithelial degeneration (arrow), and interstitial edema (Hematoxylin and Eosin; x400), (D) renal tissue of rat pre-treated with nano-composite showing minimal interstitial edema, normal nephron, and normal tubular epithelial lining. (Hematoxylin & Eosin stain; x200) and (E) renal tissue of rat post-treated with nano-composite showing normal nephron (thin arrow), normal cytoplasmic tubular epithelial lining, and minimal interstitial edema (Hematoxylin & Eosin ;x200).

Cd treatment at a dose of 2 mg/kg for thirty days revealed clear signs of toxicity with reference to decreased body weight and increased organ (liver and kidney) weights as compared to control group (Table 1). These findings are in agreement with previous reports demonstrating that Cd toxicity leads to abnormal body and organ weights³⁴ while the pre-treatment with nano-composite showed reduction in the liver and kidney weights as compared to Cd group.

Transaminases play an important role in protein and amino acid metabolism. Increased activities of serum AST, ALT, and GGT are well known diagnostic indicators of hepatic injury. In cases such as liver damage with hepatocellular lesions, these enzymes are released from the liver into the blood stream³⁵. Also ALP is considered as an enzyme of hepatocytes plasma membrane, thus an increase in serum ALP activity has been related to damage of the liver cell membranes³⁶.

As a result of the imbalance among antioxidants/ oxidants ratio in the cells, the levels of hepatic enzymes (AST, ALT, ALP and GGT) elevate in serum due to tissue necrosis or membrane damage and subsequent leakage of enzymes into the serum³⁷. Administration of CdCl₂ to rats resulted in a statistically increase in the levels of these enzymes: ALT, AST, ALP and GGT in the serum when compared with the control group. These characteristic features of Cd-induced liver toxicity are similar to those previously reported by other investigators.^{38,39} In this study, Damage to hepatic structure integrity induced by CdCl₂ is further supported by our histopathological examination, where there were focal portal infiltration by chronic nonspecific inflammatory cells rich in lymphocytes, and congested sinusoids. The hepatocytes showed focal marked eosinophilic cytoplasm with nuclear granular degenerative changes, condensed nuclear chromatin and congested dilated portal vessels. On the other hand, there was a significant decrease in the levels of these enzymes in serum of rats pre-treated with nano-composite compared to Cd group as an indication of protective effect of nano-composite against liver damage induced by Cd. However, this effect was more profound and more effective in nano-composite pre-treated group. Also, the histopathological examination of this group exhibited reduction in the pathological features of liver as compared to Cd group. Serum levels of the measured hepatic enzymes were not affected by oral administration of ZnO/Ascorbyl palmitate nano-composite in rats suggesting a safe use of this composite on liver.

Urea is the first acute renal marker which increases when the kidney suffers any kind of injury. Otherwise, creatinine is the most trustable renal marker and increase only when the majority of renal function is lost.⁴⁰ Chawla⁴¹ reported that increased levels of serum urea and creatinine are linked to kidney disease.

In current study, the Cd group showed a significant increase in serum urea and creatinine compared to the control group that might suggest the inability of the kidney to excrete these products, indicating an impairment of kidney functions. Similar observation was obtained by Novelli.⁴² It has also been proposed that Cd exerts a direct toxic effect on the glomerulus, and this leads to decrease in urea and creatinine clearance.⁴³ Cd induced tissue damage was also revealed by severe pathological changes in kidney of Cd treated rats. Kidney section revealed the presence of focal cytoplasmic tubular epithelial degeneration, interstitial edema, and

this leads to increase in serum urea and creatinine. Moreover, in the present study the pre-treatment with nano-composite significantly ameliorated the increased serum levels of creatinine and urea. This was obvious as there were minimal histological changes compared to Cd group. This suggests a potent protective effect of this composite against Cd nephrotoxicity.

Cd induced oxidative damage has been demonstrated by the increased lipid peroxidation and inhibition of enzymes required to prevent such oxidative damage.⁴⁴ The SOD, CAT, and GSH are essential parts of cellular antioxidant defense system, and they play an essential role in protection against oxidative stress.⁴⁵

In Cd treated rats, there was a significant increase in the level of TBARS in both liver and kidney, as compared to normal control. This could be possibly due to excessive formation of free radicals, which leads to deterioration of biological macromolecules³², which associated with a distinct decrease in the activity of the antioxidants SOD, CAT, GPx, and GSH in the liver and kidney of the animals exposed to Cd. Eybl⁴⁶ and Kawamoto⁴⁷ reported that Cd is thought to induce lipid peroxidation and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function.

The decrease in SOD levels may be due to inactivation of SOD by either Cd induced lipid peroxidation⁴⁸ or the antagonistic effect of Cd with copper and zinc, which are important metals for the activity of SOD molecule.⁴⁹ The reduction in the activity of CAT may be due to the accumulation of superoxide radicals and hydrogen peroxide. The activity of glutathione peroxidase enzyme was decreased as a result of Se–Cd complex formation in the active part of this enzyme.⁵⁰ The decrement in GSH content could be probably due to either increased utilization of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents, or enhanced utilization of GSH by GPx.⁵¹

It has been proposed that the enhancement of lipid peroxidation by Cd in rats is a consequence of a decrease of antioxidant enzymes.⁵²

In the present study, Rats pre-treated with nano-composite showed a significant increase in the levels of SOD, CAT, GPx, GSH, and significant decrease in the level of TBARS when compared to control group. This finding shows the ability of nano-composite to reduce reactive free radicals that might lessen oxidative damage to the liver and kidney and improve the activities of the antioxidant enzymes. This may be due to the presence of Ascorbyl palmitate which has a powerful antioxidant properties and protects cell membrane from oxidative damage^{19,20}. However, the oral administration of the nano-composite alone to rats did not change the levels of these parameters suggesting a safe use of this composite on liver and kidney.

CONCLUSION

According to the results obtained in the present study, ZnO/Ascorbyl palmitate nano-composite played a protective role against Cd toxicity in rat liver and kidney via suppression of oxidative stress. However, further studies are needed to fully elucidate the mechanism of this protection.

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