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Research Article

Protective Role of Ce Nanoparticles Against the Hepatotoxicity Induced by Exposure to Paraquat

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Abstract

Objectives: The present study aimed to investigate the antioxidant activity of cerium oxide nanoparticles (CeNPs) against paraquat (PQ)-induced liver injury in rats.

Methods: Thirty-two male rats were divided into four 8-member groups and treated intraperitoneally with PQ and/or CeNPs for 14 days. Group 1 received PQ (5 mg/kg/d), group 2 received CeNPs (15, 30, and 60 mg/kg/d), group 3 received a combination of PQ (5 mg/kg/d) and CeNPs (15, 30, and 60 mg/kg/d), and group 4 (control group) received saline solution. Serum samples along with liver tissue samples were collected from all the rats. Oxidative stress (OS) biomarkers including total antioxidant capacity, lipid peroxidation, total thiol groups, DNA damage, and nitric oxide levels were determined. Histological samples were also analyzed using hematoxylin and eosin staining slides.

Results: Levels of oxidative stress and hepatic tissue damage were significantly higher in the PQ group compared to the control group. CeNPs at a dose of 15 mg/kg showed the antioxidant activity and compromised the PQ-induced damage.

Conclusion: In the scenario tested in this study, CeNPs could reduce the levels of OS, as well as hepatic damage induced by PQ.

Keywords: Cerium oxide nanoparticle, Paraquat, Oxidative stress, Liver

Background

Paraquat (PQ) *(N, N′*-dimethyl-4,4′-bipyridinium dichloride) is regarded as one of the most broadly-used herbicides worldwide. As a fast-acting non-selective chemical, PQ breaks down the tissues of green plants and lodges inside them (1). Ingestion and contact with the skin are the most common routes of exposure to PQ. PQ triggers harmful chemical reactions in various organs including lungs, liver, and kidneys (2,3). Multiple-organ failures, mainly that of lungs (4,5), kidneys (6,7), and liver (8,9) are indicators of serious PQ poisoning. Although most studies are fixated on PQ's special oxidative lung poisoning, injuries of other organs such as hepatotoxicity are important and could be fatal. Liver is the main intrinsic antioxidant reservoir. Furthermore, because of its anatomic position, as well as the role in enzymatic metabolism and detoxification, liver is considered as a major target for oxidative damage mediated by xenobiotics. Paraclinical manifestations of PQ hepatic toxicity include liver enzymes elevation and histopathological variations (10). By interfering in the intracellular electron transfer systems of plants, PQ inhibits the reduction of NADP to NADPH through photosynthesis, thereby exerting the herbicidal activity (11). This activity results in the formation of reactive oxygen species (ROS) such as superoxide anion, singlet oxygen, and hydroxyl and peroxyl radicals (12). ROS can affect the unsaturated lipids of cell membranes and destruct plant cell organelles, and lead to cell mortality (13,14). The high death rate following PQ poisoning has been ascribed to the absence of an antidote or operative treatment to ameliorate the harmful effects of this herbicide.

Oxidative stress (OS) has been considered a probable mechanism through which PQ induces its toxic effects; in this regard, researchers along with clinicians have mainly focused on benefiting from antioxidants as a treatment modality for PQ poisoning (15,16). Cerium oxide nanoparticles (CeNPs) have shown free radical scavenging and antioxidant activities (17). Along with other lanthanide elements in nature, Ce is also found in the minerals like alanite, bastanite, monazite, cerite, and samarskite; however, only bastanite and monazite are

important sources commercially (18). The present study aimed to assess the antioxidant activities of CeNPs against PQ-induced liver injury.

Materials and Methods

Materials

CeNPs (30 nm, US Research Nanomaterials, Inc company) used in this study were supplied by Notrino compony, and PQ (99% purity) was purchased from Sigma–Aldrich (St. Louis, USA).

Animal Treatment

Thirty-two male Wistar rats (180–250 g) were obtained from the Animal Colony of the Pasteur Institute, Iran, and kept under standard environmental conditions (22 ± 1^{0}C temperature, 45%–55% humidity, and 12/12-h light/dark cycle). The animals were randomly divided into 4 groups, each containing 8 rats. They were treated intraperitoneally with PQ and/or CeNPs. Group 1 received PQ (5 mg/kg/d), group 2 received CeNPs (15, 30, and 60 mg/kg/d), group 3 received a combination of PQ (5 mg/kg/d) and CeNPs (15, 30, and 60 mg/kg/d), and group 4 (control group) received saline solution. After 24 hours, serum and liver tissue samples were collected from all the rats. The protocol of the study was approved by the Ethics Committee of the Hamadan University of Medical Sciences (No: 940118144).

Sample Collection

The rat livers were cleaned with saline solution immediately after separation and then frozen in liquid nitrogen and stored at –70°C until analysis. Liver allocations were homogenized in 1:5 volumes of PBS (pH 7.4). The resultant homogenate was then centrifuged at 3000 rpm for 10 minutes (Universal 320R, Hettich Germany). Next, the supernatant was collected and used as liver total homogenate sample. The homogenate was later centrifuged again at 3000 g for 15 minutes. The supernatant was stored at –80°C for additional biochemical assays and some parts were immersed in 10% formalin for histological studies (19).

Biochemical Analysis

Evaluation of Liver Function

The levels of liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by standard procedure of routine kits (Pars Azmoon, Iran).

Measurement of Oxidative Stress Biomarkers

Evaluation of Cellular Lipid Peroxidation

Thiobarbituric acid (TBA) was used to measure lipid peroxidation (LPO), in that TBA reacts against lipid peroxide molecules. Plasma samples were mixed with 20% trichloric acetic acid (TCA) and the resultant precipitate was dissolved in 0.05M sulfuric acid. Afterward, 0.2% TBA in 2M sodium sulfate was added and the mixture was heated at boiling temperature in water bath for 30 minutes. TBARS (thiobarbituric acid reacting substances) adducts were extracted using n-butanol and the optical density was measured at 532 nm. Reaction was performed at a low pH level (4.8) and high temperature (90°C), and the maximum optical density was measured at 532 nm (20).

Evaluation of Total Antioxidant Capacity

The total antioxidant capacity (TAC) was measured using the ferric reducing ability of plasma (FRAP) assay. This assay is based on the capacity of plasma to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ (tripyridyl-s-triazin). The interaction of Fe^{2+} and TPTZ produces a blue color complex. Maximum optical density was measured at 593 nm (21).

Evaluation of Total Thiol Molecules

To evaluate the total thiol molecules in the plasma, Dithinitrobazoic acid (DTNB) was used as the reagent. DTNB reacts with thiol functional groups and creates a yellow compound that shows a good absorbance at 412 nm (22).

Nitric Oxide Assay

Nitric oxide (NO) levels were measured by an ELISA kit (Cayman Chemical Co.).

DNA Damage Assay

The 8-hydroxydeoxyguanosine (8-OHdG) levels (DNA damage) in the liver samples were determined using an ELISA kit (Highly Sensitive 8-OHdG ELISA kit, Japan). This assay kit showed acceptable levels of sensitivity, specificity, and inter- and intra-assay accuracy. It is also suitable for analyzing small amounts of samples.

Total Protein Assay

Protein concentrations of the samples were tested using the Bradford technique by means of concentrated Coomassie blue reagent. Bovine serum albumin was also employed as the standard (23).

Histological Studies

Immediately after separation, liver (N = 3/group) tissues were submerged in 10% neutral buffered formalin solution. Liver aliquots were dehydrated in the graded concentrations of ethanol, immersed in xylene, and embedded in paraffin. Sections were cut at 5 µm thicknesses on a rotary microtome and then fixed and stained using hematoxylin and eosin. Finally, the sections were photographed with a digital camera (Nikon E800, Japan) attached to a microscope. The histological changes were studied for each rat through accessing five serial coronal sections at

400x magnification. An experienced histologist who was uninformed about the treatment conditions carried out the histological assessments (24).

Statistical Analysis

Mean and standard error were determined for all parameters. Data were analyzed in SPSS version 16.0, using one-way ANOVA test followed by the Tukey post hoc test. A *P* value <0.05 was considered statistically significant.

Results

Liver Enzymes

Figures 1a and 1b show the mean ± SE of ALT and AST in the groups. ALT and AST levels were significantly higher in the PQ group compared to all other groups. No significant differences were observed between other groups in terms of ALT and AST levels.

Total Antioxidant Capacity

A statistically significant reduction was noted in TAC for PQ group in comparison with the control group. Compared to the PQ group, a significant elevation in TAC was observed in the group receiving CeNPs (15, 30, and 60 mg/kg). No significant differences were found between the CeNPs+PQ group and PQ group in terms of TAC level (Figure 2). PQ caused a significant reduction in TAC level compared to CeNPs 30 mg/kg and CeNPs 60 mg/kg in the liver homogenates. Co-administration of CeNPs at a dose of 30 mg/kg and PQ significantly decreased PQ-reduced TAC level (Figure 3).

Lipid Peroxidation

PQ triggered a significant elevation in the serum LPO level in the PQ group compared to the control group. CeNPs at doses of 15, 30, and 60 mg/kg caused a significant reduction in the LPO level compared to the PQ. Furthermore, no significant difference was observed in the group co-administered with CeNPs and PQ compared to other groups (Figure 4a).

In the liver homogenates, PQ caused a significant elevation in LPO in the PQ group compared to the control group. CeNPs at doses of 15, 30, and 60 mg/kg caused a significant reduction in LPO compared to the PQ. Moreover, the co-administration of 30 and 60 mg/kg doses of CeNPs and PQ significantly reduced PQ-induced LPO (Figure 4b).

Total Thiol Groups

According to blood test results, PQ led to a significant reduction in total thiol group (TTG) compared to the CeNPs administered at a dose of 30 mg/kg. A significant elevation in TTG was seen in CeNPs (60 mg/kg) + PQ

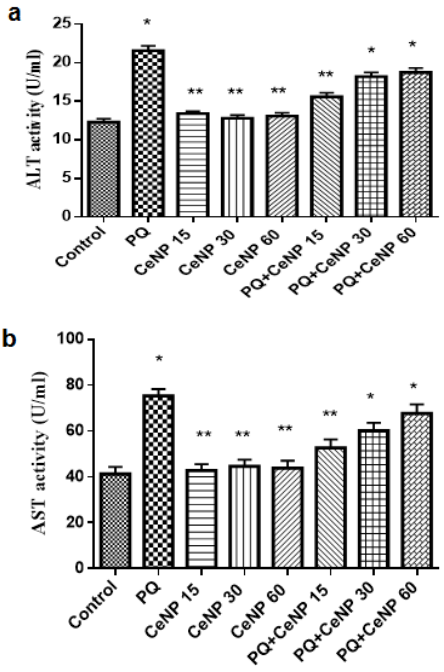

Figure 1. (**a**) Alanin Transferase (ALT) and (b) Aspartat Transferase (AST) Levels in Blood Samples Collected From Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

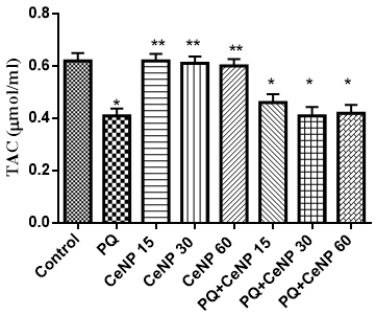

Figure 2. Total Antioxidant Capacity (TAC) in Blood Samples Collected From Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

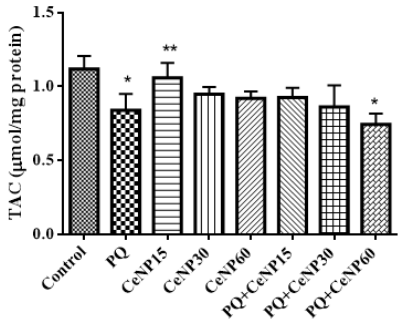

Figure 3. Total Antioxidant Capacity (TAC) in Liver Tissues of Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

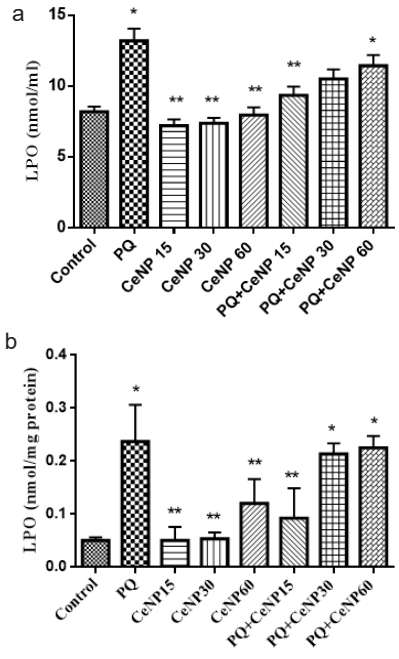

Figure 4. Lipid Peroxidation (LPO) in (**a**) Blood Samples Collected From Rats and (**b**) Liver Tissues of Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

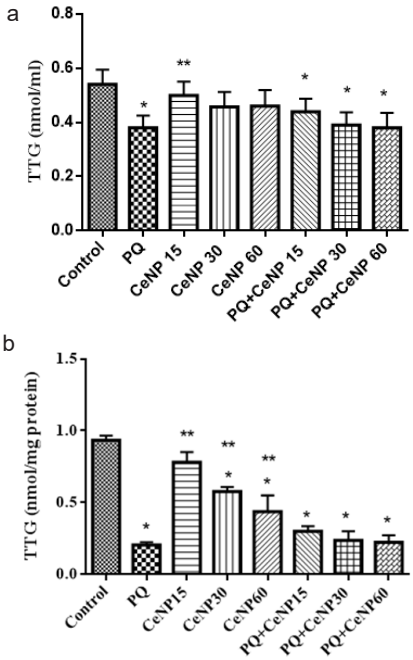

Figure 5. Total Thiol Molecules (TTM) in (**a**) Blood Samples Collected From Rats and (**b**) Liver Tissues of Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

group compared to PQ group. Moreover, no significant difference was detected between the CeNPs group and other groups (Figure 5a).

In terms of the liver homogenates, no significant differences were detected in TTG levels between the groups (Figure 5b).

DNA Damage

Regarding the liver homogenates, a significant elevation in DNA damage was seen in PQ-treated group compared to the control and CeNPs groups at doses of 15, 30, and 60 mg/kg. While, the co-administration of CeNPs at doses of 15, 30, and 60 mg/kg and PQ significantly decreased PQ-induced DNA damage (Figure 6).

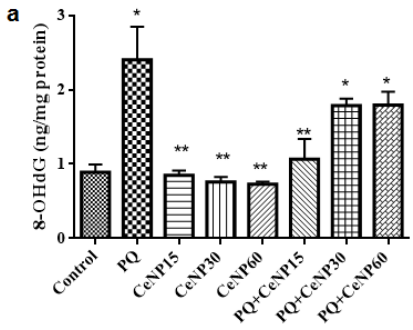

Figure 6. DNA Damage in Liver Tissues of Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

Nitric Oxide Level

Furthermore, analysis of liver homogenates displayed a significant higher NO level in the PQ group rather than the CeNPs group at a dose of 60 mg/kg. Nonetheless, no significant difference was noticed in the NO level between the CeNPs group (15 and 30 mg/kg) and the control group (Figure 7).

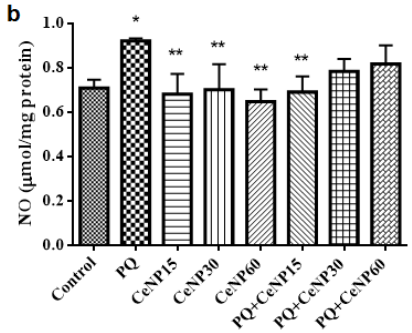

Figure 7. Nitric Oxide (NO) Level in Liver Tissues of Rats. ***Note***: PQ, Paraquat; CeNPs, Cerium oxide nanoparticles; Values are presented as mean ± SE 95% CI (n¼48). a, Significantly different from control group; *Significantly different from control group at $P < 0.05$; **Significantly different from PQ group at $P < 0.05$.

Histological Examination

Histological remarks of liver sections obtained from the normal control group revealed normal cellular architecture with well-defined hepatic cells, sinusoidal spaces and central veins (Figure 8a). The most intense damage was observed in the poisoning group (PQ group). The liver sections displayed great fatty changes, extensive infiltration

of inflammatory cells, dilated sinusoid, and the loss of cellular margins (Figure 9). In the PQ group, normal cellular architecture with a mild level of fatty change and lymphocyte infiltration was observed which was comparable to the control and CeNPs groups (Figure 9).

Discussion

The current study aimed to assess the antioxidant capacity of CeNPs in rats with PQ-induced hepatic damage. PQ induced OS in the liver and changed the levels of ALT and AST. In addition, exposure to PQ resulted in a decrement in the levels of TAC and TTG in the serum and liver homogenates. A significant increase in the enzymatic activities of AST and ALT in the blood serum indicate severe damage to the liver (25). In the present study, it was found that CeNPs modulated PQ-induced changes in serum biomarkers, liver histology, and OS level, proposing that CeNPs could protect the liver against the PQ. CeNPs can decrease ROS generation, prohibit inflammation, and maintain antioxidant enzymes and LPO content in a biological system (26). Treatment of rats with CeNPs at doses of 15 and 30 mg/kg resulted in lower levels of intracellular ROS and LPO. Since CeNPs have a wide variety of applications, further investigations are required to explain the mechanism of action of these chemicals in order to distinguish the consequence of their extensive uses (27). The results of this study showed that CeNPs at a dose of 60 mg/kg may be slightly toxic. The protective effect of CeNPs was also studied after exposure to malathion subchronic in reproductive system (28). Hence the clinical value of this study is also confirmed. CeNPs prevented hepatic damage via controlling the levels of NO, DNA damage, and oxidative injuries. In the present study, PQ induced oxidative injury in the blood and liver. Antioxidant enzymes work in a coordinated manner to prevent the oxidative damage. The metabolic function of liver in the detoxification of xenobiotics leads to the production of ROS, where enzymes such as SOD, CAT, and GPx play important roles in preventing OS in the liver tissue (29-32).

The high death rate following exposure to PQ is attributed to the lack of an antidote or operational treatment to ameliorate its harmful effects. Many instances of severe poisoning and death have been reported over the past decades (1,33). Although the ultimate mechanism of PQ toxicity has not been described, the cyclic single electron reduction/oxidation is a crucial mechanistic incident (34). With this knowledge in mind that PQ induces its harmful effects mainly through OS-induced mechanisms, researchers have focused on the use of antioxidants as an alternative for the treatment of PQ liver toxicity (24). The effect of vitamin E on toxicity caused by PQ has been shown in a number of studies. Vitamin E deficiency leads to a severe PQ toxicity in animals (35). In another study,

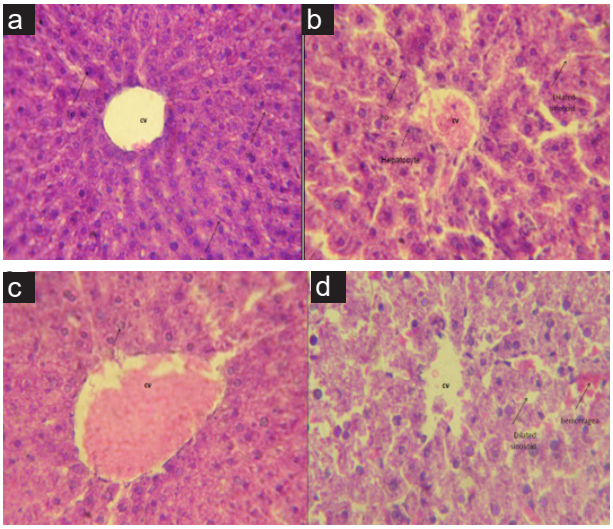

Figure 8. H&E Staining in (a) Control, (b) CeNPs 15 mg/kg, (c) CeNPs 30 mg/kg, and (d) CeNPs 60 mg/kg Groups. After treatment, livers were fixed and serially sectioned into 5 µm sections and stained with H&E.
Note: Magnification: 40x; CeNPs: Cerium oxide nanoparticles.

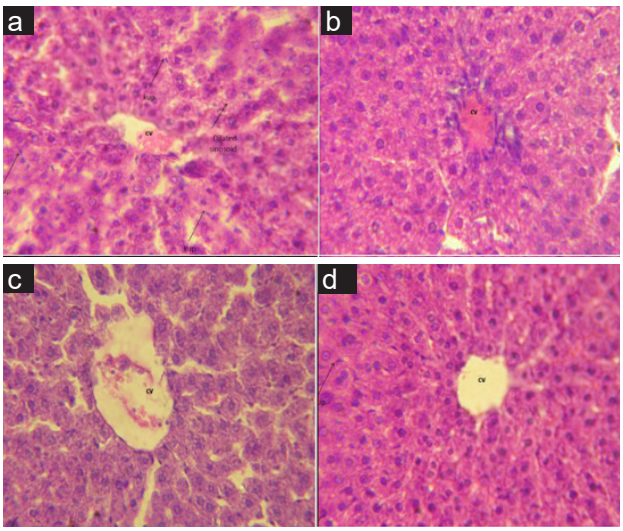

Figure 9. H&E Staining in (**a**) Paraquat, (**b**) CeNPs 15 mg/kg +PQ, (**c**) CeNPs 30 mg/kg +PQ, and (**d**) CeNPs 60 mg/kg +PQ Groups. After treatment, livers were fixed and serially sectioned into 5 µm sections and stained with H&E.
Note: Magnification: 40x; CeNPs: Cerium oxide nanoparticles.

the administration of NAC to PQ-challenged animals interrupted the PQ-induced diffusion of chemoattractants into the neutrophils in the bronchoalveolar lavage fluid and considerably reduced the infiltration of inflammatory cells, signifying that NAC can exhibit protective properties by impeding the inflammation (13,36). Newly, interest has significantly increased in discovering effective antioxidants that could be used in nutrition or medicinal chemicals to replace synthetic antioxidants, for the management of poisoning with pesticides (37,38). Moreover, exposure to CeNPs has been associated with changes in OS and inflammation induced by PQ. Furthermore, an increase in NO level is associated with the activation of macrophages in PQ toxicity.

Conclusion

In summary, according to our results, CeNPs could effectively prevent the liver damage induced by PQ in rats. However, further studies are required to clarify the pharmacological significance of its effects on PQ poisoning.

Conflict of Interest Disclosures

The authors declare no conflict of interests.

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