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RESEARCH PAPER

Cerium oxide nanoparticle modulates hepatic damage, inflammatory and oxidative stress biomarkers in a dose-dependent manner: an in vivo study of rat liver

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ABSTRACT

Objective (s): Cerium oxide nanoparticles, nanoceria (CeNPs) is a novel nanoparticle that has great potential for the treatment of various diseases. This study aimed to investigate the effects of CeNPs on oxidative stress biomarkers in the liver of male rats.

Materials and Methods: Twenty-four male Wistar rats were equally distributed into 4 groups (n=6/each). The first group was controlled and the next three groups received an intraperitoneal injection (IP) of CeNPs (15, 30 and 60 mg/kg/day) for 7 days. After treatment, serum and liver tissue were isolated. ALT and AST concentration, total antioxidant capacity (TAC), total thiol molecules (TTM), interleukin 17 (IL-17), nitric oxide (NO) and TNF-α were measured.

Results: CeNPs 30, 60 mg/kg caused a significant increased in NO (P=0.03, P=0.001), TNF-α (P=0.03, P=0.01) and IL-17 (P=0.04, P=0.01) levels, compared with the control group. Also CeNPs caused a decrease in the TTM (P=0.002) and increased malondialdehyde (MDA) (P=0.04) in 60 mg/kg group compared to the control group. CeNPs at 15 mg/kg significantly suppressed the increase in plasma activities of aminotransferases (ALT (P=0.001), AST (P=0.01)), and liver IL-17 (P=0.01) and NO (P=0.02) concentrations compared to the control group.

Conclusion: These results suggest that the effects of CeNPs are dose-dependent and at 15 mg/kg dose, it may have protective effects. Moreover, CeNPs at 30 and 60 mg/kg doses showed immunotoxicity and oxidative effects in the liver.

Keywords: *Cerium oxide nanoparticles; Liver; Oxidative stress*

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INTRODUCTION

Nanotechnology, nowadays, has obtained a considerable deal of interest due to the applications in the area of medicine and industrial [1]. Cerium oxide nanoparticles (CeNPs) not only exhibit an interesting potential in industrial use such as solar cells, gas sensors, and catalysts [2], but also possess a broad range of usage in gene therapy, drug delivery, molecular imaging and medicine. Furthermore, nanoparticles (NPs) like CeNPs are able to induce reactive oxygen species (ROS) in several cell types [3]. But, the link between CeNPs and oxidative stress has not been well studied [4, 5].

Nitric oxide (NO) is a critical mediator in the physiology and, also, pathophysiology of the liver. NO is induced in various cells of the liver, including hepatocytes, Kupffer cells and other immune cells [6]. Interestingly, produced of NO, can function as a significant source of reactive nitrogen species (RNS). In particular, peroxynitrite ($ONOO^-$), a strong biological oxidant, is responsible for damaging a broad range of cellular molecules comprising proteins, lipids and DNA. It, also, facilitates protein nitration which affects the structure and function of target proteins [7].

Liver cells and neutrophils are a source of

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proinflammatory cytokines, chemokines, ROS and RNS, which increase oxidative stress in injury induced by toxicants and ischemia/reperfusion [8]. Kupffer cells release cytotoxic mediators, like ROS, and proinflammatory mediators, such as cytokines and chemokines. Cytokines can induce adhesion molecule and chemokine formation in liver, which in turn is modulated by oxidant stress [9].

Interleukin 17 (IL-17), which has, recently, gained much attention in the field of immunology, is produced by a distinct type of T cell named T helper 17 cells. This cytokine plays essential regulatory roles and its increased levels associated with cases including a development of inflammation, autoimmunity and tumors. Additionally, the high levels of IL-17 are associated with increased levels of cytokines such as TNF-α [10, 11]. A spectrum of tissue responses such as cell activation, generation of ROS, inflammation and cell death can be elicited by these NPs [12]. Cytotoxicity of CeNPs may be, partially, due to their induction of cellular oxidative stress via the production of free radicals and ROS [13]. This subject is of clinical significance because certain pathological situation such as inflammation are associated with enhanced oxidative stress and, in turn, this may alter the sensitivity of cells and tissues to potentially cytotoxic effect of CeNPs [14, 15]. Therefore, this study aimed to examine the potency of CeNPs in different doses on inflammation and oxidative stress status in rat liver.

MATERIALS AND METHODS

Reagents and chemicals

Ethylene-diamine-tetra-acetic acid (EDTA), coomassie blue, bovine serum albumin (BSA), 2, 4, 6 Tri pyridyl-s-tiazine (TPTZ), dithiobis-(2-nitrobenzoic acid) (DTNB), Tris base were obtained from Sigma. The CeNPs (100 nm) used in this study were supplied by Neutrino company (Milan, Italy). TNF-α kit (Ransel kit, Randox Laboratories Ltd, Crumlin, UK), Interleukin 17 (Oxis Research, USA) and NO kit (ZellBio, Germany), were used in this study. The nanoparticle was suspended in deionized water.

Animals and treatments

Adult male Wistar rats weighing 220–250 g were maintained under standard laboratory conditions (12-h dark/light cycles at 22 ± 2 °C). Prior to the initiation of the experiment, an ethical clearance was obtained from the Iranian Animal Ethics Society and the local university to perform the experiments on animals ethically, approved by the Research Committee of Hamadan University of Medical Sciences, Iran. Animals at random distributed into 4 groups of six animals and treated for one week intraperitoneally (IP). The groups were as follows: control group (animals received only normal saline), CeNPs group (was received 15, 30, 60 mg/kg/day).

Experimental protocols

Preparation of liver homogenate

At the end of the experiment, 24 hours after the last dose, fasted rats were anesthetized with ketamine (50 mg/kg) and serum samples were collected and stored at −20 °C. Liver tissue was removed and minced with a small scissor in a cold mannitol solution containing 0.225 M D-mannitol, 75 mM sucrose, and 0.2 mM ethylene-diamine-tetra-acetic acid (EDTA). The minced liver was gently homogenized, in a homogenizer with a Teflon pestle, and then centrifuged. The supernatant was used to evaluate the parameters.

Liver function evaluation, TNF-α and IL-17 assay

Serum ALT and AST were assayed by the Pars Azemoon kit. Liver levels of TNF-α and IL-17 were determined using High-sensitivity sandwich enzyme-linked immunosorbent (ELISA) technique (TNF-α and IL-17 ELISA kit; eBioscience (affymetrix, Inc, USA)) according to the manufacture's instruction.

Oxidative stress biomarkers

Measurement of liver nitric oxide (NO)

Liver NO content was assayed calorimetrically (ZellBio GmbH, Ulm-Germany), according to the manufacturer's instructions.

Measurement of liver total antioxidant capacity (TAC)

It was measured by the ferric reducing ability of plasma method (FRAP), which is based on the ability of plasma to reduce Fe^{3+} to Fe^{+2} in the presence of TPTZ. The reaction between Fe^{2+} and TPTZ gives a complex with blue color and the maximum absorbance at 593 nm [16].

Measurement of liver total thiol molecules (TTM)

To evaluate the plasma total thiol molecules, DTNB was used as a reagent. DTNB reacts with

thiol-containing molecules and generates a yellow complex which has a good absorbance at 412 nm in spectrophotometer [17].

Measurement of liver malondialdehyde (MDA)

Contents of MDA in plasma, urine and saliva were measured spectrofluorometrically as Thiobarbituric acid (TBA) reactive substances. TBA reacts with MDA and is formed TBA reactant substances (TBARs), as biomarkers of oxidative damage to polyunsaturated fatty acids and measured at 532 nm by spectrophotometer [18].

Total protein

Protein concentration in the samples was measured by the Bradford method using concentrated Coomassie blue reagent. Also, bovine serum albumin was used as standard [19].

Statistical analysis

Results were expressed as Mean±SE. All data were analyzed with SPSS (Version: 16) and GraphPad Prism version 6.0 (GraphPad Software, San Diego-USA). One-way ANOVA followed by post hoc Tukey's test was used to detect the statistical significance between groups. Differences between groups were considered significant if P<0.05.

RESULTS

Hepatic damage biomarkers in the serum

As seen in Table 1, CeNPs 15 mg/kg group significantly decreased the ALT level, compared to control group (P=0.001) (87.2±2.5 v.s. 118.5±4.4). CeNPs 15 mg/kg reduced the AST level, as the same as that of ALT, compared to control group significantly (P=0.01) (32.2±2.2 v.s. 51.5±3.5).

Table 1. Hepatic damage and Inflammation biomarkers in liver and serum of rat

Group / parameter	ALT(U/L)	AST(U/L)	TNF-α(pg/ml)	IL-17(pg/ml)
Control	118.5 ± 4.4	51.5±3.5	52.25 ± 4.9	79.2 ± 7.2
15 mg/kg/day	87.2±2.5 [b]	32.2±2.2 [b]	49.2 ± 2.3	48.8 ± 4.7 [b]
30 mg/kg/day	112.8± 5.5	48.4±3.6	75.6 ± 3.2 [a]	100.4 ± 5.3 [a]
60 mg/kg/day	120.8± 7.2	54.6±3.8	107.6± 3.2 [b]	127.3 ± 11.8[b]

Inflammation biomarkers in the liver

CeNPs with the doses of 30 (P=0.03) and 60 (P=0.01) mg/kg significantly increased the TNF-α level, (75.6±3.2 and 107.6±3.2 v.s. 52.25±4.9), respectively, as compared to control. Although CeNPs 15 mg/kg caused a decrease in the TNF-α level, however, that was not statistically significant (Table 1). Also CeNPs 30 (P=0.04) and 60 (P=0.01) mg/kg proved a significant increase in the IL-17 level of animals in comparison to control group (100.4±5.3 and 127.3±11.8 v.s.79.2±7.2). Moreover, NPs with dose of 15 mg/kg caused reduce in the liver IL-17 level, significantly (P=0.01) (48.8±4.7 v.s. 79.2±7.2) (Table 1).

Oxidative stress-related biomarkers in the liver

NO levels of each group are illustrated in Fig 1. As clearly shown, groups receiving CeNPs with doses of 30 (P=0.03) and 60 mg/kg (P=0.001) indicated significantly elevated NO levels (159.4 ± 4.6 and 215.8 ± 5.9 v.s. 121.7±4.2) respectively, compared to control. Additionally, group receiving 15 mg/kg revealed a significant decreased level of NO (P=0.02) (83.6 ± 8.8 v.s. 121.7 ± 4.2).

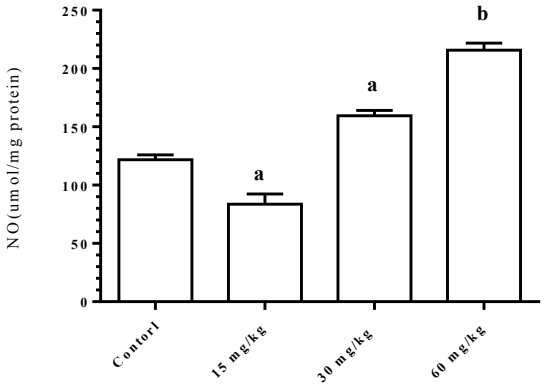

Fig 1. Hepatic NO level. Values are the Mean±SE for each group. NO: Nitric oxide; CeNPs: Cerium oxide nanoparticles. [a]Significantly different from control group at p<0.05. [b]Significantly different from control group at p< 0.01

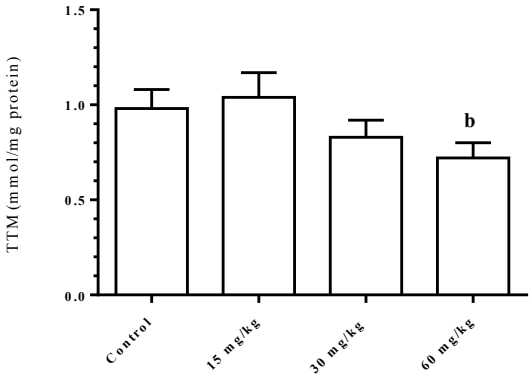

Fig 2. Hepatic TTM level. Values are the Mean±SE for each group. TTM: Total thiol molecules; CeNPs: Cerium oxide nanoparticles. [a]Significantly different from control group at p<0.05. [b]Significantly different from control group at p<0.01

It also shows the TTM levels in different groups, where CeNPs 60 mg/kg led to a significant (P=0.002) decrease (0.72±0.08 v.s. 0.98±0.10), compared to control. Other two groups (CeNPs 15 and 30 mg/kg), were not significantly different compared to control group (Fig 2).

Furthermore, serum TAC levels of rats at the end of the experiments are shown in Fig 3. As observed, there were no significant differences between the control and CeNPs-treated groups. Also CeNPs (15 mg/kg) treatment decreased liver MDA content compared to control rats (87.1±6.15 v.s. 117.1±7.14) (P=0.04) (Fig 4).

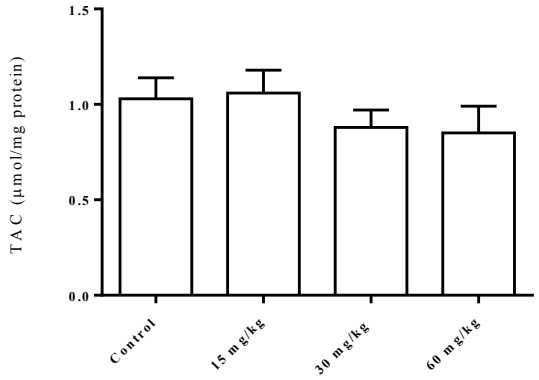

Fig 3. Hepatic TAC level. Values are the Mean±SE for each group. TAC: Total antioxidant capacity; CeNPs: Cerium oxide nanoparticles. [a]Significantly different from control group at p<0.05. [b]Significantly different from control group at p<0.01

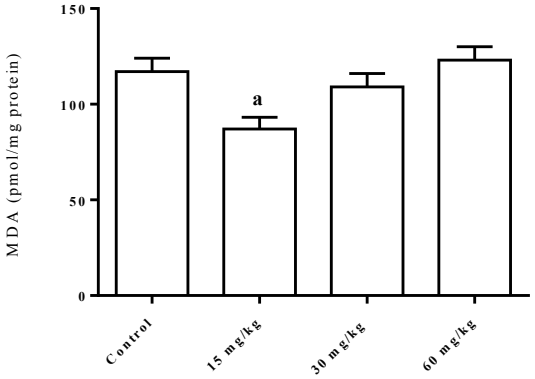

Fig 4. Hepatic MDA level. Values are the Mean±SE for each group. MDA: Malondialdehyde; CeNPs: Cerium oxide nanoparticles. [a]Significantly different from control group at p<0.05. [b]Significantly different from control group at p<0.01

DISCUSSION

Nanotoxicity studies associated with various NPs have attracted intense research interest due to the broader applications of NPs in our daily lives. The purpose of this study was to determine the roles of CeNPs, which there have been lots of paradox reports of its oxidant/antioxidant properties [20]. There has been reported many medical applications for CeNPs such as anticancer [21, 22], free radical scavenger [23], inhibitor of oxidative stress, nuclear factor-κB and apoptosis [4, 13]. In the contrary, some studies have found that CeNPs induce cytotoxicity and oxidative stress [24]. Liver, as the center of body detoxification, is constantly faced with endogenous or exogenous deleterious compounds. High levels of ALT and AST are known to indicate hepatocyte damage [25, 26]. Findings of this work showed that CeNPs 15 mg/kg, by decreasing ALT and AST levels, illustrated benefit effects which might be considered as a hepatoprotective. However, the other two doses showed damage and toxic properties on hepatocytes. Ibrahim et al. have reported that CeNPs (40 mg/kg) increased liver enzyme (ALT and AST) activity [27].

NO is involved in the regulation of several physiological systems [28]. NO, also, seems to be a key regulator of the hepatocyte bile salt pool [29]. Furthermore, it is involved in the release of some pro-inflammatory mediators like TNF-α [30]. Additionally, excess RNS and NO production could damage hepatocytes and activate hepatic satellite cells, which play a central role in liver damage [28, 31]. Our results in liver homogenate demonstrated that CeNPs decreased the oxidative/nitrosative toxic stress at dose of 15 mg/kg, as shown by decreased NO and increased levels of TTM and TAC. Decreased NO level at 15 mg/kg dose of CeNPs may suggest hepatoprotective effects this NPs. Along with our study, Rollin et al [32], also suggested a role for CeNPs in the formation of disulfide bridges in biomolecules containing thiol. But for dosed of 30 and 60 mg/kg, depletion of thiol-contained molecules can result in oxidative/nitrosative stress which is thought to contribute to hepatocyte damage. NO generated from Kupffer cells, RNS and cytokines is also involved in this process [31, 33]. The results of the present study showed that at 60 mg/kg dose of CeNPs TTM significantly decreased compared with that of control group. Studies have shown that decrease in TTM pool (such as cysteine thiol and glutathione [GSH], is closely related to the pathogenesis of liver diseases such as chronic alcohol intake [34], drug-induced liver toxicity [35], biliary cirrhosis [36] and nonalcoholic fatty liver disease [37]. Interestingly, the results of this study showed increased level in MDA in rats receiving 60 mg/kg dose of CeNPs

compared to control group. The mechanism of ROS production by nanoparticles is not well understood. Eom et al. have suggested that CeNPs may induce toxic effects. It is generally assumed that toxicity increases as the nanoparticles size becomes smaller [38].

In line with the results of all above-mentioned parameters, CeNPs caused similar effects on immune-related mediators examined in this study. IL-17 increases in conditions like inflammation, autoimmunity and tumors [11]. Also, CeNPs at 15 mg/kg decreased significantly IL-17 level in the liver. Moreover, TNF-α level displayed the same response to CeNPs 15 mg/kg treatment. Thus, it can be regarded as an immune-modulatory factor which decreases inflammation. In contrast with these findings, 30 and 60 mg/kg doses showed increased IL-17 and TNF-α levels that are known to develop inflammation and autoimmune disorders.

Taken together, the present study provides the first evidence that CeNPs at 15 mg/kg act as an antioxidant that prevents oxidative stress and suppresses inflammatory responses. However, this study has represented that CeNPs (30 and 60 mg/kg) caused an immunotoxicity in the liver tissue. Since CeNPs are used in an increasing number of applications [39], therefore, further studies are required to illustrate the mechanism of action of these compounds.

CONCLUSION

The results of the present study indicate that the effects of CeNPs are dose-dependent and at 15 mg/kg dose, it may have anti-inflammatory and antioxidative effects in the liver of rats. Based on our results, CNPs (30 and 60 mg/kg) caused several adverse effects such as alters in liver enzyme activities, the generation of ROS and inflammatory factors.

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