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The Pivotal role of Cerium oxide nanoparticles in thioacetamide induced hepatorenal injury in rat.

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Abstract

The objective of the present study is to investigate the protective effect of cerium oxide nanoparticles (15mg, 30mg/kg) on hepatorenal injuries induced by thioacetamide (100mg/kg). The size of cerium oxide nanoparticles (CeO2NPs) was 50nm. We evaluated the protective effect of CeO2NPs by studying their effect on oxidative stress, inflammatory markers, and histopathological changes in the liver and kidney. CeO2NPs improved antioxidant status in liver or kidney as manifested by enhancement of reduced glutathione (GSH) and a reduction in malondialdehyde (MDA), Nitic oxide (NO), and oxidized glutathione (GSSG). Also, CeO2NPs mitigated the decrease in plasma catalase (CAT), total antioxidant capacity (TAC), and hepatorenal ATP content resulting from thioacetamide (TAA). Moreover, the treatment of rats with CeO2NPs + TAA alleviated the significant increase in plasma liver enzymes (Gamma-glutamyltransferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase(ALP)), kidney function parameters (Creatinine, urea, uric acid) and inflammatory markers (C-reactive protein (CRP), tumor necrosis factor-alpha(TNF-α), interleukin-6(IL-6)). The protective effect of ZnO-NPs was affirmed by histopathological study of the liver and kidney. Our results proposed that CeO2NPs may relieve TAA-hepatorenal toxicity via their antioxidant and anti-inflammatory properties that participated in the suppression of oxidative stress.

Keywords: Cerium oxide nanoparticles, Hepatorenal injuries, Oxidative stress, Inflammatory markers, Histopathology.

1. Introduction

Liver diseases are chronic and widespread worldwide. Hepatic fibrosis is a persistent toxic reaction that is associated with substantial morbidity and death. Chronic hepatic damage causes hepatic fibrosis. Extracellular matrix (ECM) buildup disturbs the natural architecture of the liver, causing fibrosis. Hepatic activated stellate cells (HSC) are the main source of excess collagen. After a fibrogenic stimulation, HSCs go from dormant to active[1]. Tumor necrosis factor-α (TNF-α) is one of the cytokines released in inflammation that results in liver fibrosis by activating local HSCs and turning them into fibrogenic myofibroblasts[2,3]. Excessive ROS production, which leads to mitochondrial oxidative stress, apoptosis, and cell injury, is another pathway involved in liver fibrosis [4,5]. Thioacetamide (TAA) is a hepatotoxin that has been shown to cause hepatorenal injuries in animals [6]. It had previously been reported that there is a link between liver disease and renal failure in cirrhosis patients [7].

The nanoparticle has a beneficial action that cannot be produced by conventional therapy due to the inability of the latter to deliver an adequate concentration of therapeutic agents into the liver and most organs [8–10]. CeO_2NPs compared to other nanometallic oxides, have the property of being antioxidants since their shift from the reduced to the oxidized state is reversible and the process may be restarted [11]. Besides CeO_2NPs have a potent antioxidant properties, its medicinal uses as antibacterial and neuroprotective are reported due to

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its ability to retain higher surface area because of its small crystal size [12–14]. Despite debates about CeNPs toxicity, some reports resolved the controversy where the CeO_2NPs toxicity and efficacy depend on its synthesis [15].

The acceptable issue for hepatorenal dysfunction is the good control for prevention of disease progression leading to the liver or kidney failure, and cirrhosis. So, our aim is to demonstrate the biological activity of CeO_2NPs in the mitigation of TAA- induced hepatotoxicity and nephrotoxicity.

2. Experimental

2.1. Material

2.1.1 Drug and chemicals

TAA and Cerium oxide nanoparticles were purchased from "Sigma-Aldrich, Co., USA". The size of CeO2NPs was 50nm. Methanol (HPLC grade) was secured from Loba Co, India. Perchloric acid was bought from Loba Co, India. Sulphosalsilic acid and P-amino benzyl glutamate and pyrogallol were purchased from TMMEDIA Co, India. The 1, 1, 3, 3-tetraethoxypropane, glutathione (GSH), and oxidized glutathione (GSSG) were gotten from Sigma Aldrich (USA). Other used chemicals were of purity grade and were bought from known supplies.

2.2. Animals

Adult albino Wistar rats (150–200 gram) were purchased from the National Research Centre (Cairo, Egypt) and given a normal laboratory diet with tap water available as needed. The experimental animals were kept in an air-conditioned room with equal time of light/dark cycle at ambient temperature. The study methods were carried out in accordance with the "Medical Research Ethics Committee (MREC)" at the "National Research Centre's ethical standards" for the care and use of experimental animals, which were approved by the "MREC" (Reg. No. 19218). Also, according to the national and international ethical guidelines stated by the bioethics committee of NRC that comply with the (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

2.3. Experimental design

After two weeks as an acclimatization period, animals were randomly divided into four groups (n=8) as follows. Group 1: received same volume of vehicle and aided as normal control. Group 2: received TAA (100 mg/kg) intraperitoneally three times per week for six weeks [16]. Groups 3 and 4: received CeO2 NPs (15 and 30 mg/kg suspended in distilled water, IP) [17] daily for six weeks with TAA. CeO2 NPs were injected half an hour before TAA injection.

2.4. Collection of Blood Samples and tissue homogenates.

At the end of the experimental period, all animals fasted overnight and blood samples were taken from the retro-orbital vein under diethyl ether anesthesia [18]. After settling, blood was centrifuged for 15 min at 3000 rpm (Sigma Laborzentifugen, 2K15, Germany) and separated plasma was stored in Eppendorf tubes at -30˚ for biochemical analysis. Animals were killed by cervical dislocation under ether anesthesia. Liver and kidney were immediately removed, washed in cooled saline. A weighed part of each tissue was homogenized with cooled saline (0.9% NaCl) to prepare homogenate. The homogenate was then centrifuged at 3000 rpm for 10 min. at 5°C using a cooling centrifuge and the supernatant was kept at -80 ◌C[19].

2.5. Hepatorenal function and lipids tests

The obtained plasma was used to estimate the levels of AST, ALT, ALP, GGT, total cholesterol, triglycerides, glucose total protein, albumin, urea, creatinine and uric acid using kits produced by Salucea Company.

2.6. Oxidative stress parameters

2.6.1. In plasma

Antioxidant parameters, MDA, GSH, SOD, catalase and total antioxidant capacity (TAC) were evaluated calorimetrically using kits from by "Elabscience Biotechnology Kit (Building B18, Biomedical Park, #858 Gaoxin Road, Donghu Hi-Tech Development Area, Wuhan, Hubei, P.R.C)".

2.6.2. In liver and kidney homogenates

Hepatic and renal content of GSH, GSSG, NO, MDA, and ATP were evaluated by HPLC system of "Agilent HP 1200 series (USA)[20–23].

2.7. Inflammatory markers

Plasma TNF-α, IL-6 and CRP Elisa kits were purchased from "Sunlong Biotech Co., Ltd, China".

2.8. Histological Examination.

Liver and kidney samples from several groups of rats were fixed in 10% buffered neutral formalin and processed for paraffin slices. The specimens were washed in tap water, dehydrated in ethyl alcohol serial dilutions, clarified in xylene, and lastly embedded in paraffin. Haematoxylin and eosin stain was used to stain 4–5 mm thick paraffin blocks (H&E) [24].

2.9. Statistical Analysis.

All values are provided as means ± standard error (SE). This study's data were analysed using "one-way ANOVA followed by Tukey's as a multiple comparisons test". These statistical tests were performed using "Graphpad Prism 8 (Inc., San Diego, USA)". $p \leq 0.05$ was judged significant.

3. Results

3.1. Liver enzymes

The present results revealed that induction of hepatotoxicity by TAA elevated plasma levels of ALT, AST, ALP and GGT, as compared to the normal control group (Table 1). CeO_2NPs low dose treatment significantly reduced plasma levels of ALT, AST, ALP and GGT by 220, 238, 289 & 95 % respectively, the dose-dependent efficacy has appeared where treatment with CeO_2NPs high dose significantly reduced plasma levels of ALT, AST, ALP and GGT by 401, 392, 487 and 146 % respectively as compared with TAA-intoxicated groups (Table 1).

The synthetic capacity of liver is affected by TAA injection where TAA-induced hepatotoxicity reduced albumin and total protein by 61 and 32% respectively as compared to normal control group but this capacity significantly improved by treatment with CeO_2NPs at dose-dependent manner as follow 36 and 18% respectively for low dose and 60 and 29% respectively for high dose as compared with TAA-intoxicated group.

3.2. Kidney function

The present results indicated a marked upsurge in levels of plasma uric acid, urea and creatinine of rats treated by TAA (Table 2) as compared to control group. While in the groups treated with TAA+low or high dose of cerium nanoparticles, the levels of plasma uric acid, urea and creatinine were significantly reduced. The values of CeO_2NPs high dose were significantly less than those of low dose.

3.3. Lipid profile and glucose

The present results revealed that induction of hepatotoxicity by TAA elevated plasma levels of TC, TG and reduced glucose level, as compared to normal control group (Table 3). Conversely, the administration of CeO_2NPs at low or high dose to TAA -intoxicated rats significantly reduced plasma levels of TC, TG and elevated glucose levels. High dosage CeO2NPs have higher impact than low dose.

3.4. Oxidative stress parameters

3.4.1. In Plasma

The results illustrated in Fig. 1 showed that the administration of rats with TAA led to a momentous increase in levels of SOD, GSH and MDA while TAC and CAT levels decreased significantly as compared to control group. Alternatively, the administration of rats with TAA + CeO_2NPs at low or high doses has significantly reduced levels of GSH, MDA and SOD and elevated significantly TAC and CAT levels Compared to TAA-intoxicated groups, in a dose-dependent way.

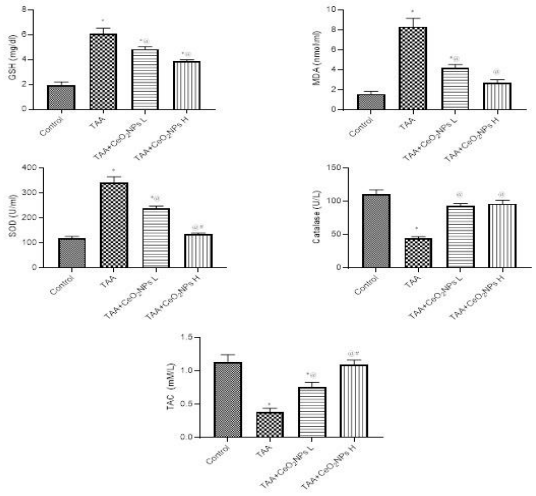


Fig.1 Effect of cerium oxide nanoparticles on oxidative stress parameters of rats treated with thioacetamide. Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test. (* vs control group, $^{@}$ vs TAA group and $^{\#}$ vs TAA+CeO_2 NPs L group) at p<0.05. L: 15mg/Kg, H: 30mg/Kg

3.4.2. In liver and kidney homogenates

The injection of TAA resulted in a significant elevation in both hepatic and renal content of MDA, GSSG and NO as well as a significant depletion in content of reduced glutathione and ATP as compared to the control group (Figs 2, 3). In the TAA+ CeO2NPs (low or high dose) groups, the hepatic and renal oxidative stress parameters were improved as evidenced by a reduction in MDA, GSSG and NO contents and enhancement of GSH and ATP levels as compared with TAA- intoxicated groups. The results of correlation (Fig4) indicated a positive correlation between hepatic MDA and plasma liver enzymes. Also, there is a positive correlation between hepatic MDA and plasma kidney function parameters.

Table 1. Effect of cerium oxide nanoparticles on liver function of rats treated with thioacetamide

Groups	ALT (U/l)	AST (U/L	GGT (U/L)	Albumin (g/dL)	ALP (U/L)	Total Protein(g/dL)
control	11.13±2.90	10.75±3.11	16.38±3.93	3.85±0.62	87.88±13.28	7.29±0.58
TAA	70.38±12.28*	63.88±14.22*	50.63±11.94*	1.51±0.53*	539.90±60.68*	4.96±0.55*
TAA+CeO_2 NPs L	45.86±5.58$^{*@}$	38.29±5.16$^{*@}$	35.00±4.58$^{*@}$	2.91±0.35$^{*@}$	285.60±28.35$^{*@}$	6.24±0.21$^{*@}$
TAA+CeO_2 NPs H	25.71±5.62$^{*@\#}$	21.71±6.24$^{@\#}$	26.71±4.75$^{*@}$	3.83±0.63$^{@\#}$	111.90±11.28$^{@\#}$	7.04±0.44$^{@\#}$

Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. (* vs control group, $^{@}$ vs TAA group and $^{\#}$ vs TAA+CeO_2 NPs L group) at p<0.05. L: 15mg/Kg, H: 30mg/Kg.

Table 2. Effect of cerium oxide nanoparticles on kidney function of rats treated with thioacetamide

Groups	Creatinine (mg/dL)	Uric Acid (mg/dL)	Urea (mg/dL)
control	0.57±0.12	5.14±0.84	43.76±8.04
TAA	0.98±0.16*	9.55±1.36*	98.59±9.74*
TAA+CeO_2 NPs **L**	0.78±0.09$^{*@}$	7.11±0.91$^{*@}$	64.37±7.29$^{*@}$
TAA+ CeO_2 NPs **H**	0.71±0.13$^{@}$	5.41±1.03$^{@\#}$	49.50±4.91$^{@\#}$

Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. (* vs control group, $^{@}$ vs TAA group and $^{\#}$ vs TAA+CeO_2 NPs L group) at $p<0.05$. L: 15mg/Kg, H: 30mg/Kg.

Table3. Effect of cerium oxide nanoparticles on plasma lipid profile and glucose of rats treated with thioacetamide.

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
control	71.44±6.14	79.21±12.33	101.50±9.70
TAA	197.90±13.66*	172.60±20.49*	63.09±5.79*
TAA+CeO_2 NPs **L**	96.31±8.03$^{*@}$	96.41±8.35$^{@}$	97.01±10.01$^{@}$
TAA+ CeO_2 NPs **H**	82.71±6.58$^{@\#}$	88.13±8.72$^{@\#}$	97.37±13.87$^{@}$

Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. (* vs control group, $^{@}$ vs TAA group and $^{\#}$ vs TAA+CeO_2 NPs L group) at $p<0.05$. L: 15mg/Kg, H: 30mg/Kg

3.5. Plasma inflammatory markers

Injection of TAA results in a substantial elevation in TNF-α, IL-6, and CRP (642, 259 and 369 % respectively) as compared to the control group (Fig.5). On the contrary, the treatment of rats with TAA and CeO2NPs at low or high doses significantly reduced the elevation of inflammatory markers as compared to TAA group. There is a positive correlation between hepatic MDA and plasma inflammatory markers (Fig.6).

3.6. Histopathological result

3.6.1. Histological results of liver:

Histopathological examination of liver tissues treated with TAA only showed the hepatic architecture was disorganized with marked affection of the hepatocytes in the form of cirrhotic nodules , fibrous tissue run in septa between the hepatocytes lobules forming pseudolobules, hyperchromatic nuclei with clumped chromatin(High grade dysplasia), and some apoptotic cells with hypereosinophilic cytoplasm ,pyknotic and fragmented nuclei. Many blood vessels were obviously congested, while some hepatocytes swelled or other appear with binucleated cells. Macrovesicular steatosis was noticed and the cytoplasm of some hepatocytes was replaced by bubbles of fat. Most of the blood sinusoids appeared narrow or obliterated. Moreover, Cytoplasmic hydropic degeneration and many of hepatocytes with vacuolated cytoplasm were observed in TAA group (Fig7A&B&C&D). The histological sections of rats in the group treated with TAA and subjected to low dose of cerium oxide nano particles revealed well preserved of hepatic architecture in the form of no cirrhotic nodules no fibrosis no apoptotic cells no dysplasia ,most hepatocytes appeared normal, the hepatocytes are arranged in cords but some hepatocyte appeared swelling , others cells were necrotic or binucleated cells. Thickening of portal vein vascular wall, fibrotic and hyperplasia of bile duct, dilated congested central vein were also detected in the previous group (Fig8A&B). In the case of rats treated with TAA and subjected to high dose of cerium oxide nano particles, some improvement in pathological changes in the form of regeneration of normal hepatic features were noticed although the congestion, dilation of portal vein , hyperplasia of the bile duct, aggregates of lymphocytic inflammatory cells, thickening of portal vein vascular wall , and mild fibrosis. Also, necrotic changes in hepatocytes, hyperchromatic /pyknotic nucleus and some apoptotic cells with hypereosinophilic cytoplasm were observed in TAA+ CeO2NPs high dose group beside congested portal vein and aggregation of inflammatory cells (white arrow). (Fig8C&D).

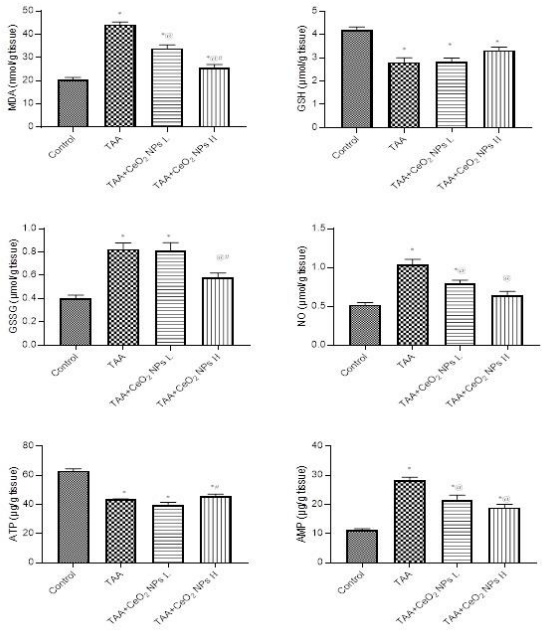


Fig.2. Effect of cerium oxide nanoparticles on hepatic oxidative stress and energy parameters of rats treated with thioacetamide. Each value

represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test. (* vs control group, @ vs TAA group and # vs TAA+CeO_2 NPs L group) at $p<0.05$. L: 15mg/Kg, H: 30mg/Kg

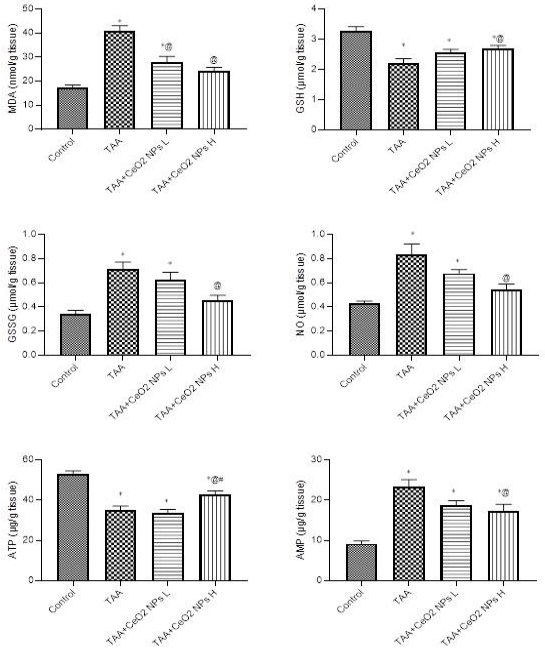


Fig.3. Effect of cerium oxide nanoparticles on renal oxidative stress and energy parameters of rats treated with thioacetamide. Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test. (* vs control group, @ vs TAA group and # vs TAA+CeO_2 NPs L group) at $p<0.05$. L: 15mg/Kg, H: 30mg/Kg

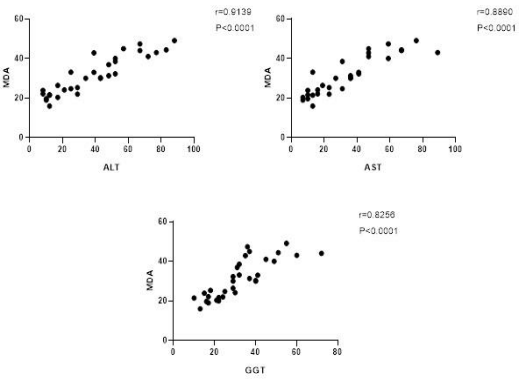


Fig.4 Correlation between hepatic MDA and plasma liver enzymes

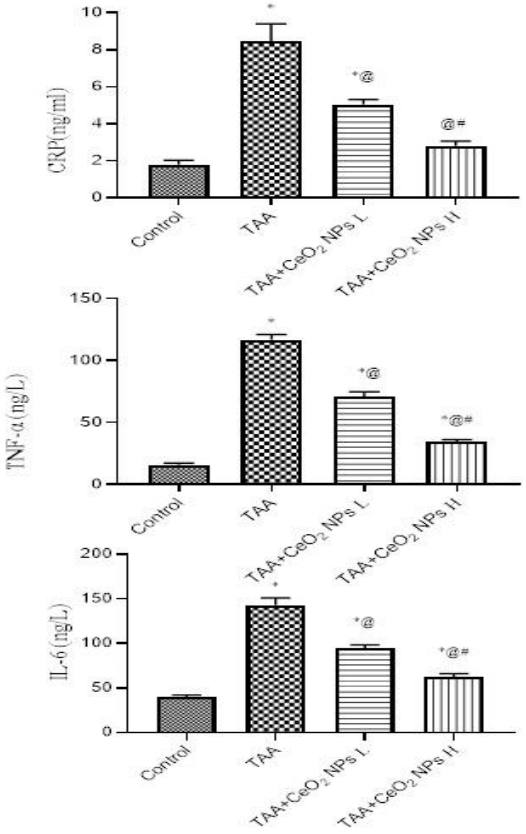


Fig.5. Effect of cerium oxide nanoparticles on plasma inflammatory markers of rats treated with thioacetamide. Each value represents the mean of 8 animals ± SE. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test. (* vs control group, @ vs TAA group and # vs TAA+CeO_2 NPs L group) at $p<0.05$. L: 15mg/Kg, H: 30mg/Kg

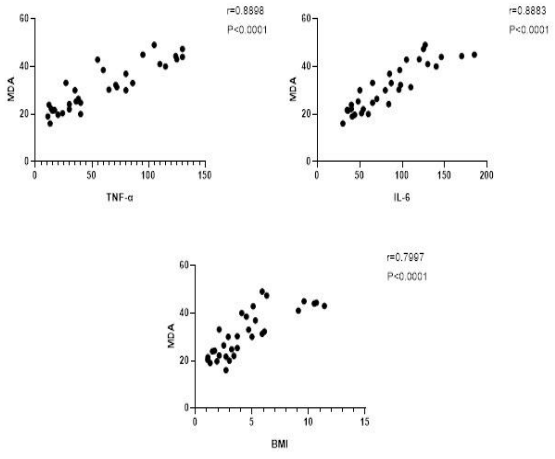
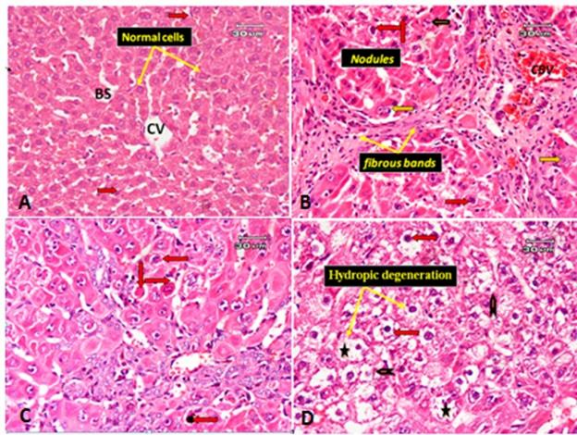


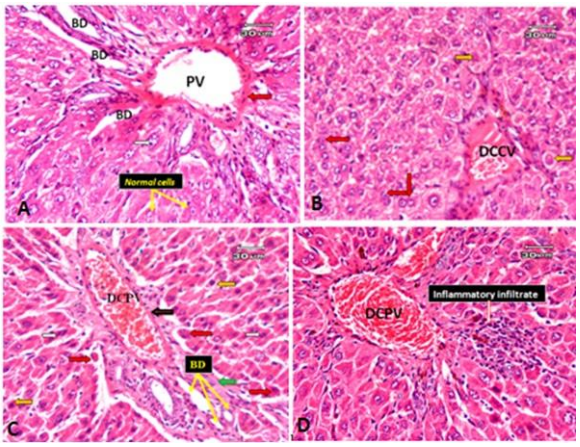
Fig.6 Correlation between hepatic MDA and plasma inflammatory markers



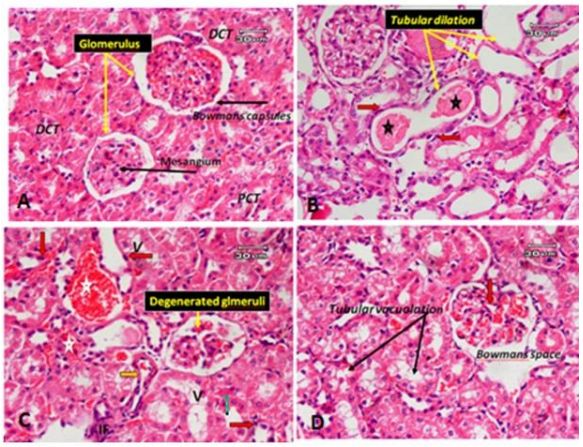
Fig(7): Photomicrographs of rat liver(A) Normal control showing normal hepatic architecture, central vein (CV), blood sinusoids (BS), kupffer cells (red arrow) and endothelial cells, (B) liver section of rat treated with TAA only showing cirrhotic nodules, thick fibrous bands formed many fibroblast, and the collagen fiber (yellow arrow), hyperchomatic nuclei with clumping chromatin(dysplasia) (red arrow), some of the cells are binucleated (orange arrow), and others swelling (black arrow) and congested of many blood vessel(CBV),(C) liver section of rat treated with TAA only(another filed) showing liver hepatocytes apoptosis, (D) liver section of rat treated with TAA only(another filed) showed macrovesicular steatosis (star), pyknotic cells (red arrow), most of the blood sinusoids appeared narrow or obliterated (black arrow).

3.6.2. Histological results of kidney:

Histopathological examination of kidney tissues treated with TAA only showed some tubular necrosis with dilatation in the lumen of tubules with epithelial flattening, hyaline casts are present in the medullary of some tubules. Degenerated glomeruli with intraglomerular hemorrhage, increased Bowmans space and hemorrhage in interstitium were found in TAA sections. The Kidney section of TAA group is characterized by Atrophy of some tubules, few inflammatory infiltrations, cytoplasmic vacuolation and the presence of vacuoles in the renal tubular epithelium (Fig9A&B&C&D). The sections of rats in the group treated with TAA and subjected to low dose of cerium oxide nano particles (Fig.10 A) showing good improvement in histological changes induced by TAA in the form of no hyaline cast, no degeneration of glomeruli although cytoplasmic vacuolation in some renal tubular epithelium, thickening of brush of some tubules and narrowing of lumen. Pathological examination of kidney sections concerned rats treated with TAA and high dose of cerium oxide nano particles, revealed some improvement in pathological changes in the form of normal distal and proximal tubule, but some glomeruli appeared shrinkage with cytoplasmic vacuolation and an increased bowman space (Fig. 10B & C).

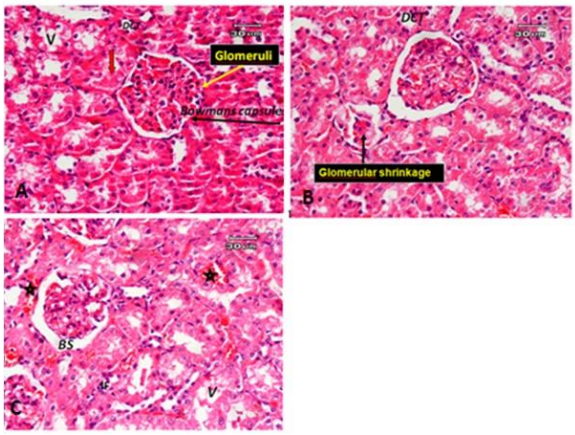


Fig(8): Photomicrographs of rat liver(A)liver section of rat treated with TAA and a low dose of cerium oxide nanoparticles showed well preserved of hepatic architecture, most hepatocytes appeared normal, although some hepatocyte appeared swelling(white arrow), thickening of portal vein vascular wall and fibrotic(red arrow). Hyperplasia of the bile duct(BD), (B) liver section of rat treated with TAA and subjected to cerium oxide nanoparticles at a low dose(another filed) showed dilated and congested central vein (DCCV), some binucleated cells(red arrow) with a few necrotic cells(orange arrow),(C) liver section of rat treated with TAA and subjected to cerium oxide nanoparticles at high dose showed some improvement in pathological changes although congested portal vein(DCPV), (D) liver section of rat treated with TAA and subjected to cerium oxide nanoparticles at high dose (another filed)showed dilate congested portal vein and aggregates of inflammatory cells(white arrow).



Fig(9): Photomicrographs of rat kidney(A)Normal control showing proximal convoluted tubules(PCT), distal convoluted tubules(DCT), (B) kidney section of rat treated with TAA only showing some tubular necrosis with extensive

tubular dilatation, epithelial flattening, (red arrow)hyaline casts are present in the medullary of some tubules(star),(C) kidney section of rat treated with TAA only (another filed) showing degenerated glomeruli with intraglomerular hemorrhage (yellow arrow), hemorrhage in the interstitium (star), and atrophic of some tubules (orange arrow), few inflammatory infiltrate(IF),(D) kidney section of rat treated with TAA only (another filed) showing vacuoles in renal tubule epithelium(black arrow), increase Bowman's space and interglomerular hemorrhage(red arrow).



Fig(10): Photomicrographs of rat kidney(A) kidney section rat treated with TAA and a low dose of cerium oxide nanoparticles showed good improvement in pathological changes in the form of normal glomeruli with Bowman's capsule and distal tubules but cytoplasmic vacuolation in some renal epithelium (V), and narrowing lumen(red arrow),(B) kidney section of rat treated with TAA and subjected to cerium oxide nanoparticles at high dose showed improvement in pathological changes in the form of the normal distal and proximal tubule. However, some glomeruli appeared shrinkage,(C) Another field in the kidney of TAA+CeNPs high dose group showed increased bowman space(BS) and few mononuclear inflammatory cells(IF), vascular congestion in interstitial tissue (star).

4. Discussion

In this study, the treatment of rats with nanometallic cerium oxide (CeO2NPs) reduced oxidative stress, inflammation, and hepatorenal pathology induced by Thioacetamide (TAA). In the current study, the administration of rats with TAA led to hepatorenal toxicity assured by the significant increase in plasma ALT, AST, GGT, ALP, creatinine, uric acid, and urea[25]. Moreover, TAA induced histopathological changes as fibrosis, steatosis and narrowing blood sinusoids in liver or degeneration of some glomeruli with intraglomerular hemorrhage, increased Bowmans's space and interstitium hemorrhage in kidney. The histopathological changes can be attributed to an increase of hepatic or renal oxidative stress as evidenced by a significant increase in MDA, and nitric acid contents and a decrease of GSH content. The sulphydryl group on GSH makes it an important endogenous antioxidant that protects cells from ROS and detoxifies external chemicals [26] The results indicated a positive correlation between hepatic MDA and liver enzymes or between renal MDA and creatinine or uric acid levels. MDA is a lipid peroxidation marker and an indicator of oxidative stress [25]. MDA is thought to be one of the primary chemical processes involved in oxidative damage to cell structures and cell death [27]. Hydroperoxides and their aldehyde derivatives limit protein synthesis [28]. As a manifestation of oxidative stress cascade due to liver toxicant, plasma level of MDA and SOD markedly elevated which was previously reported [18,29], who also displayed the relation between increased production of free radicals and progression of liver cirrhosis. Another report also implied the link between the nephrotic syndrome and elevated level of MDA.[30].

TAA is one of the potent hepatotoxin liberating radicals where a single dose could produce centrilobular hepatic necrosis, and chronic administration led to cirrhosis [31,32]. It is reported that the main mechanism of TAA toxicity is due to oxidative stress, manifested as lipid peroxidation, protein oxidation and alterations in tissue antioxidants [33]. This mechanism dragged into a cascade of cellular degenerative changes [5] followed by as leakage of cellular cytosol (elevated ALT, AST) the specific mirror of hepatocellular degeneration [24]. Also, plasma ALP level elevated due to defective hepatic excretion as a reflection of hepatobiliary and hepatocellular injury [34,35]. These structural changes and oxidative damage could also affect the secretory function of liver leading to hypoproteinemia and hypoalbuminemia which in turn leads to circulatory and renal dysfunction[36].

CeO2 nanoparticles have exhibited CAT- and SOD-like action to cut down oxygen radicals and hydrogen peroxide levels, they also act as scavengers for ROS and for nitric oxide radical [37,38]. In the current investigation, CeO2NPs reduced oxidative stress evoked by TAA as evidenced by the decrease in hepatorenal MDA, GSSG and nitric oxide contents and enhanced the GSH level in liver or kidney [27]. Also CeO2 NPs enhanced TAC and catalase levels in blood of TAA- treated rats. Total antioxidant capacity is used to measure the antioxidant action to free radicals generated in disease [39]. The improvement of both hepatic and renal functions of TAA -group treated by CeO2NPs can be attributed to a decrease in oxidative stress. It was reported that the treatment of rats with CeO2NPs at doses 15 mg/kg lowered renal oxidative stress [40]. Furthermore, CeO2NPs prevent the decrease in ATP content of liver and kidney or a decrease in plasma glucose. During the early stages of

cirrhosis, hepatocytes are unable to sustain high levels of energy production via glycolysis due to increasing mitochondrial respiration impairment [41]. The kidney is one of the most energy-consuming organs in the human body. Kidney dysfunction is linked to a decrease in cellular ATP [42].

CeO_2NPs have a protective effect against oxidative damage and they able to scavenge oxygen free radicals and hence effectively protect mammalian cells against damage [43]. In addition, CeO2NPs offer many active sites for free radical scavenging because of their large surface/volume ratio and also the mixed valence states for unique redox chemistry [44]. Furthermore, the free radical scavenging property of CeO2NPs is regenerative, which is not the case for other antioxidants [42]. It was documented that CeO2NPs have a protective role in acetaminophen - induced liver injury[45].

Also, a big correlation was previously reported between oxidative stress due to TAA leading to chronic liver disease and deficiency of functional LDL receptor causing hypercholesteremia [46] which explain the increment of lipid profile in the present study and other chronic treatment with TAA. Here, CeO2NPs reduced the significant elevation of plasma cholesterol and triglycerides due to TAA treatment. Also, it is reported that CeO2NPs have a significant role in reduction of blood lipids in fipronil-induced hepatic steatosis [47] and in non-alcoholic fatty liver disease[48]

The histopathological results indicated the decrease of hepatic fibrosis in rats treated with TAA+ CeO2NPs. It appears that CeO2NPs protect the liver against TAA-induced fibrosis via reducing hepatic lipid peroxidation [49] or inflammatory markers implicated in the pathophysiology of different inflammatory hepatic disorders [50,51] .

It was concluded that CeO2NPs have anti-inflammatory effects in peritonitis caused by Staphylococcus epidermidis infection [52]. ROS internally and externally contribute to stellate cell activation, persistent inflammation (TNF-α, IL-6) [53], apoptosis [54], and hepatic fibrosis [55]. Moreover, CeO_2NPs inhibit the increase of hepatic nitric oxide that has a pivotal role in cellular inflammation and liver damage [56]. CeO_2NPs could improve kidney function by decreasing renal oxidative stress. It was reported that CeO_2NPs with anti-oxidant and anti-apoptotic properties attenuate renal injury evoked by Cyclophosphamide [57].

It is worthy to mention that, CeO_2NPs (50, 100, and 200 mg/kg) did not affect liver enzymes, liver, or renal tissues. CeO2NPs are safe for medicinal usage and appear to protect against oxidative stress [58]. Administration CeO2NPs in current study resulted in 1. Retain optimal antioxidant capacity by improving GSH, 2. Decrease the elevated NO level caused by TAA intoxication, 3. The more prominent action was the control and limitation of cytokines release (CRP, TNF-α and IL-6). So, it is suggested that the main mechanisms by which CeO_2NPs attain hepatorenal damage were a decrease of oxidative stress and inhibition of inflammatory mediators as well as control of NO release or preserving hepatorenal ATP content. Controlling oxidative stress or inflammatory markers may be offer a promising strategy for liver fibrosis therapy. As an antioxidant, CeO_2NPs can treat illnesses caused by unregulated oxidative stress.

5. Conflicts of interest

The authors declare that there is no conflict of interest.

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