ORIGINAL ARTICLE
Turk J Surg 2022; 38 (3): 255-265

Anti-inflammatory effects of oral and intraperitoneal administration of cerium oxide nanoparticles on experimental hepatic ischemia-reperfusion injury

Akile Zengin[1], Açelya Erikçi[2], Gökçen Telli[3], Bülent Gümüşel[4], Kemal Kösemehmetoğlu[5], Gülberk Uçar[6], Mustafa Cem Algın[7]

1 Clinic of Gastrointestinal Surgery, Malatya Training and Research Hospital, Malatya, Türkiye
2 Department of Biochemistry, Lokman Hekim University Faculty of Pharmacy, Ankara, Türkiye
3 Department of Pharmacology, Hacettepe University Faculty of Pharmacy, Ankara, Türkiye
4 Department of Pharmacology, Lokman Hekim University Faculty of Pharmacy, Ankara, Türkiye
5 Department of Pathology, Hacettepe University Faculty of Medicine, Ankara, Türkiye
6 Department of Biochemistry, Hacettepe University Faculty of Pharmacy, Ankara, Türkiye
7 Department of General Surgery, Kütahya Health Science University, Kütahya, Türkiye

ABSTRACT

Objective: Hepatic ischemia-reperfusion (IR) injury occurs in liver surgery, resection, and transplantation. Reactive oxygen species (ROS) produced following IR starts the cascade of cell damage, necrosis/apoptosis, and proinflammatory responses by activating intracellular signaling cascade to drive hepatocellular damage. Cerium oxide nanoparticles (CONPs) act as anti-inflammatory and antioxidant agents. Thus, we evaluated the protective effects of oral (o.g.) and intraperitoneal (i.p.) administration of CONPs on hepatic IR injury.

Material and Methods: Mice were randomly divided into five groups: control, sham, IR protocol, CONP+IR (i.p.), and CONP+IR (o.g.). Mouse hepatic IR protocol was applied to the animals in the IR group. CONPs (300 µg/kg) were administered 24 hours before IR protocol. Blood and tissue samples were taken after the reperfusion period.

Results: Hepatic IR injury markedly increased enzyme activities, tissue lipid peroxidation, myeloperoxidase (MPO), xanthine oxidase (XO), nitrite oxide (NO), and tissue nuclear factor kappa-B (NF-κB) p65 levels, plasma pro-inflammatory cytokines, chemokines, and adhesion molecules while decreasing antioxidant markers and caused pathological changes in hepatic tissue. The expression of tumor necrosis factor alpha (TNF-α), matrix metalloproteinase 2 (MMP-2), and 9 increased, and tissue inhibitor matrix metalloproteinase 1 (TIMP-1) expression decreased in the IR group. Pretreatment with CONPs o.g. and i.p. 24 hours before hepatic ischemia improved the biochemical parameters above and alleviated the histopathological findings.

Conclusion: Results of the present study demonstrate a significant reduction in liver degeneration by administering CONPs via i.p. and o.g. route in an experimental liver IR model, suggesting that CONPs have the extensive potential to prevent hepatic IR injury.

Keywords: Ischemia reperfusion, cerium oxide nanoparticles (CONPs), mouse, oxidative stress, inflammation

INTRODUCTION

Hepatic ischemia-reperfusion (IR) injury is a significant complication of liver-related surgical interventions, liver transplantation, liver resection, hemorrhagic shock, and liver trauma surgery, implicating a complex cascade of cellular and humoral events leading to severe cellular injury (1,2). Although several factors are involved in hepatic IR injury, such as anaerobic and aerobic metabolism, intracellular calcium overload, oxidative stress, as well as events related to neutrophils, Kupffer cells, cytokines, and chemokines (1), there is no effective prevention or treatment found yet. Thus, new therapeutic options for preventing or alleviating hepatic IR injury during liver surgery are needed.

The early phase of hepatic IR injury comprises the period less than two hours after reperfusion, which includes oxidative stress and inflammation, while the late phase, in which neutrophil accumulation and hepatocellular injury are involved, occurs at six to 48 hours after reperfusion (2). Kupffer cells, the liver macrophages, are the source of reactive oxygen species (ROS) production in the initial phase of IR injury. In contrast, hypoxia and adenosine triphosphate (ATP) loss also contribute to the ischemic period by forming ROS and activating neutrophils to produce in-

Cite this article as: Zengin A, Erikçi A, Telli G, Gümüşel B, Kösemehmetoğlu K, Uçar G, et al. Anti-inflammatory effects of oral and intraperitoneal administration of cerium oxide nanoparticles on experimental hepatic ischemia-reperfusion injury. Turk J Surg 2022; 38 (3): 255-265.

Corresponding Author
Akile Zengin
E-mail: dr.akile.zengin@gmail.com

Received: 30.12.2021
Accepted: 10.06.2022
Available Online Date: 19.09.2022

DOI: 10.47717/turkjsurg.2022.5620

flammatory cytokines, further enhancing hepatocyte injury (3). Antioxidants are used to prevent excess ROS formation in the treatment of hepatic IR injury (4); these agents do not favorably target the liver.

Recent advances in nanotechnology offer promising agents that can effectively scavenge ROS and exhibit anti-inflammatory effects (5). Among these, cerium oxide nanoparticles (CONPs), also called nanoceria, are used in the biomedical field to treat pathologies involving oxidative stress and inflammatory processes (6). CONPs act superoxide dismutase (SOD) and catalase (CAT) like activities (7). Recent studies have demonstrated that the antioxidant capacity of CONPs is 9-fold higher than that of known antioxidants (5). Recently, CONPs have been shown to block interleukin-6 (IL-6) and tissue inhibitor of metalloproteinase-2 (TIMP-2) gene overexpression in cirrhotic rats (8). In addition, studies have shown that CONPs are not toxic in therapeutic doses, relatively stable, and accumulate in the liver by administration (9).

In this study, our aim was to assess the constructive effects of CONPs on oxidative and inflammatory processes observed during IR liver injury in a mouse hepatic IR model, considering the promising benefits of CONP-based therapies.

MATERIAL and METHODS

Animal Studies

The present study was performed in compliance with the Ethical Guidelines for Animal Studies and conducted with the permission of local animal ethics committee (2017/04.02). Eight to twelve week-old male mice weighed 20-30 g were used. Ketamine (100 mg/kg) and xylazine (10 mg/kg) were used (intraperitoneally-i.p.) for anesthesia in fasted mice (16 h). Following a midline laparotomy, the liver was exteriorized, and to induce approximately 70% liver ischemia in the left lateral and median lobes, blood flow was interrupted with an atraumatic clip during 45 minutes (Figure 1). The liver and intestine were kept moist, the mice kept warm at 37°C with a heating blanket. After the ischemia period, the clamp was removed, the abdomen was sutured and the mice were recovered, thus reperfusion was provided during five hours. The mice were sacrificed under anesthesia [ketamine (500 mg/kg) and xylazine (50 mg/kg) i.p] after the reperfusion period, and tissue and blood samples were obtained. Two part of the liver samples were weighed immediately. The part to be used in histopathological analyzes was placed in 10% formalin. The second part of the samples was kept at -80°C until biochemical analysis. Blood samples were obtained by cardiac puncture and put into heparinized tubes for centrifugation at 3.000 x g for 10 min. The supernatants were stored at -20°C until biochemical experiments.

Five different group of animals were used: control, sham, IR protocol, CONP+IR (i.p.), and CONP+IR [oral gavage (o.g.)]. Each group consisted of six mice. Animals in the control group received an isovolumetric vehicle of 5% dextrose solution and then were sacrificed; blood and tissue samples were obtained. In the sham group, 5% dextrose solution was administered, and mice abdomens were just opened without clamping of the liver; blood and tissue samples were taken after the reperfusion period. Hepatic IR protocol was applied in the IR group after the vehicle administration. CONPs (300 µg/kg) in 5% sterile dextrose solution was administered 24 hours before IR protocol by i.p. injection or o.g. in CONP+IR (i.p.) and CONP+IR (o.g.) groups; blood and tissue samples were taken after the reperfusion period.

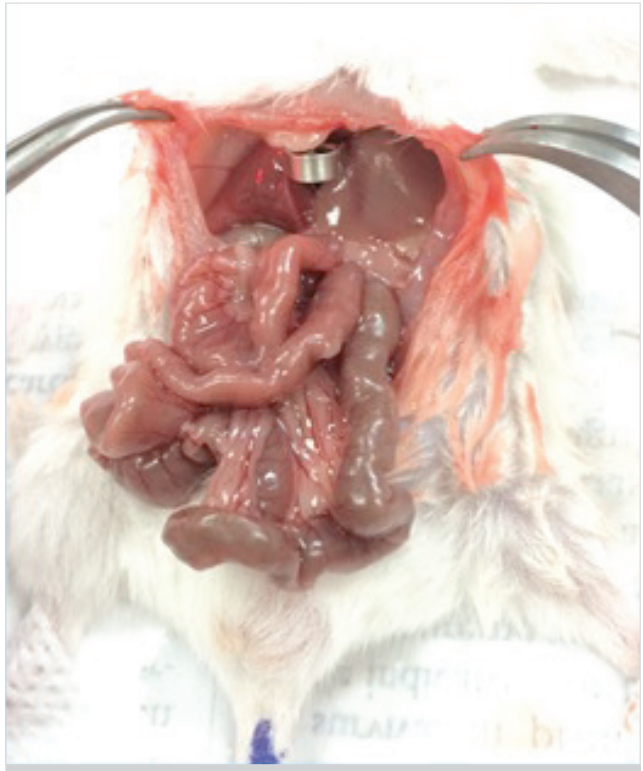

Figure 1. Partial mouse liver IR model.

The extent of liver edema was evaluated by liver/body weight (LBW) ratio. Mice in all groups were weighed. At the time of sacrifice, livers of the study groups were removed and weighed. The study groups' wet LBW was compared with that of the sham group.

Liver tissues removed from the rats were fixed in 10% formaldehyde solution for at least one day and embedded in paraffin at room temperature for 24 h. The sections of 4 µm thickness were subjected to hematoxylin & eosin staining and routine tissue monitoring. The sections were evaluated with a light microscope equipped with an image analysis program by a pathologist who was unaware of the treatment protocols. Presence of necrosis, sinus dilatation-congestion, venous congestion, inflammatory infiltration, and hepatocyte vacuolization/degeneration was considered as histopathologic evidence of liver damage (10).

Biochemical Analyses

Hepatic Enzyme Levels

Plasma transaminase and lactate dehydrogenase (LDH) levels were estimated as the markers of liver damage during hepatic IR. Plasma LDH activity was measured using an enzyme-linked immunosorbent assay (ELISA) kit (SIGMA-ALDRICH, L DH Activity Assay Kit, cat. No: MAK066); plasma alanine and aspartate aminotransferase (ALT, AST) levels were estimated using ELISA kits (SIGMA-ALDRICH, ALT Activity Assay Kit, cat. no: MAK052 and AST Activity Assay Kit, cat. no: MAK055, respectively) according to manufacturer's instructions. Results were expressed as U/L.

Oxidative Stress Parameters

Tissue malondialdehyde (MDA) level, as the indicator of lipid peroxidation, was estimated using ELISA kit (SIGMA-ALDRICH, Lipid Peroxidation (MDA) Assay Kit, cat. no: MAK085). Results were expressed as nmol/mg protein.

Tissue reduced glutathione (GSH) and oxidized (GSSG) glutathione contents were measured using glutathione assay kits (Cayman Chemicals, cat. no: 703002). Results were expressed as nmol/mg protein. GSH/GSSG ratio was calculated.

Antioxidant enzyme activities in hepatic tissue samples were determined using ELISA kits according to the manufacturer's instructions. SIGMA-ALDRICH SOD assay kit 19160 for superoxide dismutase (SOD) measurement; Glutathione Peroxidase Cellular Activity Assay Kit CGP1 for glutathione peroxidase (GPx) measurement; Glutathione S-Transferase (GST) Assay Kit CS0410 for GST measurement; Glutathione Reductase Assay Kit GRSA for glutathione reductase (GR) measurement and catalase assay kit CAT100 for catalase (CAT) measurement were used. Enzyme activities were expressed as nmol/mg protein for SOD, CAT, and GR and U/mg protein for GST and GPx.

Myeloperoxidase (MPO) Activity

Tissue MPO activities were measured using a commercially available ELISA kit (SIGMA-ALDRICH, MPO Colorimetric Activity Assay Kit, cat. no: MAK068). MPO activity was expressed as U/mg protein.

Xanthine Oxidase (XO) Activity

Since XO is the primary source of ROS at reperfusion, XO activity in liver tissues was estimated using an ELISA assay kit (SIGMA-ALDRICH, XO Activity Assay Kit cat. no: MAK078). XO activity was expressed as nmol/mg protein.

Nitric Oxide (NO)

Since it is suggested that NO mediates tissue injury during IR, reduces the harmful effects of endothelin, and improves microcirculation, liver tissue NO level was estimated using ELISA assay kit (ABNOVA, Nitric Oxide Assay, cat. no: KA1641). NO level was expressed as μM.

Tissue Nuclear Factor Kappa-B (NF-κB) p65

Since the activation of NF-κB was associated with IR injury and in vivo administration of NF-κB inhibitors reduced IR injury, in the present study, liver tissue NF-κB p65 level was determined using ELISA assay kit (MyBioSource, mouse NF-kappaB p65 ELISA Kit, catalog number MBS2508000). NF-κB p65 level was expressed as ng/mg protein.

RNA Isolation and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using the Trizol separation method (Invitrogen). 1 μg total RNA was transcribed to cDNA with a high-capacity cDNA reverse transcription kit (Supercript II). Real-time PCR was carried out using SYBR Green PCR Master Mix (Biorad, Canada) and 100 nM of F and R primers. The relative quantity of each mRNA was normalized to the relative quantity of Glyceraldehyde 3-phosphate dehydrogenase. Primer sets used in RT-PCR experiments indicated in Table 1.

Plasma and Tissue Protein Contents

Plasma and tissue protein contents were determined using a total protein assay kit (SIGMA Total Protein kit, Micro TP0100). Protein concentration was expressed as mg/mL.

Plasma Cytokine, Chemokine, and Matrix Metalloproteinase (MMP) Levels

Since it has been reported that cytokines and chemokines contribute to the pathology of IR injury, and migration of polymorphonuclear neutrophils into damaged tissue during ischemia is facilitated by endothelial expression of adhesion molecules like intercellular adhesion molecule-1 (ICAM-1), plasma cytokine and chemokine levels were determined. IL-1-α, IL-1 β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, tumor necrosis factor-α (TNF-α), and

Table 1. Primer sets used in RT-PCR experiments

GAPDH F: 5′ - CATCACTGCCACCCAGAAGACTG -3′ R: 5′- ATGCCAGTGAGCTTCCCGTTCAG -3′
TNF-α F: 5′ - GGTGCCTATGTCTCAGCCTCTT -3′ R: 5′ - GCCATAGAACTGATGAGAGGGAG -3′
MMP-2 F: 5′ - CAAGGATGGACTCCTGGCACAT -3′ R: 5′- TACTCGCCATCAGCGTTCCCAT -3′
MMP-9 F: 5′ - GCTGACTACGATAAGGACGGCA -3′ R: 5′- TAGTGGTGCAGGCAGAGTAGGA -3′
TIMP-1 F: 5′ - TCTTGGTTCCCTGGCGTACTCT -3′ R: 5′- GTGAGTGTCACTCTCCAGTTTGC -3′
F: Forward primer, R: Reverse primer.

interferon-γ (IFN-γ) levels were estimated using ELISA Multiplex assay kit (Invitrogen ProcartaPlex™ Multiplex Immunoassay) according to the manufacturer's instructions. Cytokine levels were expressed as pg/mL. Plasma IL-8 level was determined using ELISA assay kit (MyBioSource Rat IL8 ELISA Kit cat. no: MBS025179. IL-8 level was expressed as pg/mL. Plasma ICAM-1 level was measured using an ELISA assay kit (Thermo Fisher Scientific, Rat ICAM-1 ELISA Kit, ERICAM1). ICAM-1 level was expressed as pg/mL. Plasma MMP-2 and MMP-9 levels were determined using Mouse MMP 2 ELISA Kit (MyBioSource cat. no: MBS454416) and Mouse MMP 9 ELISA Kit (MyBioSource cat. no: MBS720876). MMP levels were expressed as pg/mL. Plasma TIMP-1 level was determined using ELISA assay kit (ABCAM mouse TIMP1 SimpleStep Cat. No. ab196265). TIMP-1 level was expressed as pg/mL

Statistical Analysis

All results were expressed as mean ± standard error of mean (SEM) of at least three independent experiments and analyzed by Prism 6.0 software for MacOS. Mann-Whitney U test and Kruskal-Wallis were used for comparison of groups of the variables. Correlations between variables were assessed with Pearson's correlation coefficients (r), and $p< 0.05$ was considered statistically significant.

RESULTS

Effects of CONPs on Liver Edema

Liver edema was assessed by the LBW ratio. LBW was significantly increased in the IR group compared to that of the sham group. However, administration of CONP orally and intraperitoneally significantly inhibited IR-induced increase in hepatic LBW ratio ($p< 0.01$) (Figure 2).

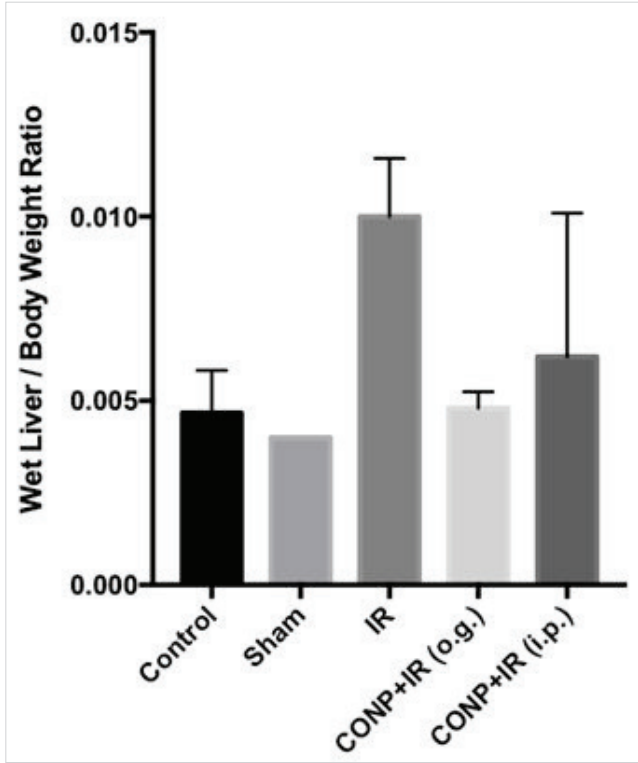

Figure 2. Liver/body weight ratio in the study groups. Results are presented as mean ± SEM (n= 6). (**p< 0.01 vs. Sham; ΨΨp< 0.01 vs. IR groups).

Effects of CONPs on Histopathological Findings

No histopathological findings were found in the liver tissue of the sham group (Figure 3A). On the contrary, the IR group showed histological changes reflecting severe hepatocellular injuries, such as loss of liver parenchyma due to hepatocyte necrosis accompanying severe polymorphonuclear leukocyte infiltration (Figure 3B), cytoplasmic vacuolization, and sinusoidal congestion. These histopathological findings were found to be highly consistent with our biochemical findings (plasma LDH, ALT, AST activities; tissue MDA content). However, morphological appearance significantly returned to almost normal in CONP+IR (i.p.) and CONP+IR (o.g.) groups (Figures 3C, 3D, respectively), indicating that treatment with CONPs ameliorates liver injury that occurred during hepatic IR in mice.

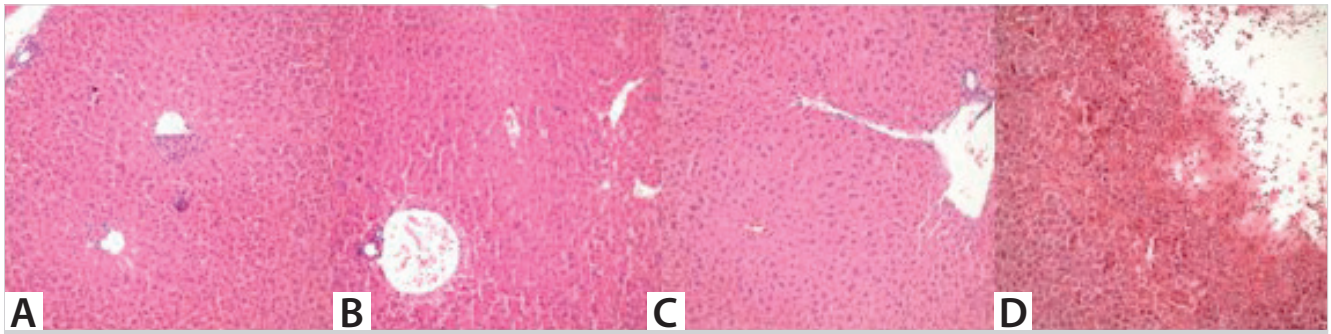

Figure 3. Hepatic histological changes in hematoxylin&eosin-stained liver sections of study groups (Original magnification: ×200). **(A)** Sham group: No pathological changes were observed. **(B)** IR group: Severe hepatocyte necrosis leading to loss of liver parenchyma with accompanying neutrophils was seen. **(C)** CONP+IR (i.p.) group: Almost normal liver parenchyma with minimal sinusoidal and venous congestion. **(D)** CONP+IR (o.g.) group: Few small necro-inflammatory foci and moderate sinusoidal congestion were present. There is no massive necrosis seen in the IR group.

Effects of CONPs on Hepatic Enzyme Levels

Plasma LDH and transaminase levels are estimated as liver damage markers during hepatic IR. Plasma LDH levels were almost four times increased in the IR group than the sham group, while CONP administration led to a significant decrease in these levels (p< 0.001). No statistically significant difference was observed between the administration of CONPs by i.p. or o.g. However, pretreatment with CONP via intraperitoneally significantly reduced the enzyme levels almost towards the levels of the sham group, which demonstrates that i.p. administration of CONPs may be helpful to reverse the tissue damage that occurred in hepatic IR (Table 2). Plasma AST and ALT activities were significantly increased after hepatic IR, and CONP administration markedly decreased the plasma levels of these liver injury indicators (p< 0.001) (Table 2). No statistically significant difference was observed between the administration of CONPs by i.p. or o.g. in terms of hepatic enzyme levels.

Table 2. Biochemical parameters in plasma and hepatic tissue samples of the study groups

	Control	Sham	IR	CONP+IR (i.p.)	CONP+IR (o.g.)
Plasma LDH (U/L)	516.11 ± 60.64	519.89 ± 42.87	1950.24 ± 183.85 ***	517.44 ± 61.17 ^^^	538.69 ± 50.54 ^^^
Plasma ALT (U/L)	19.55 ± 3.04	19.82 ± 5.58	95.55 ± 7.87 ***	20.73 ± 5.95 ^^^	22.44 ± 4.25 ^^^
Plasma AST (U/L)	38.57 ± 9.47	42.23 ± 5.69	316.97 ± 39.40 ***	43.86 ± 9.37 ^^^	46.82 ± 9.39 ^^^
Tissue MDA (nmol/mg protein)	6.56 ± 0.69	6.52 ± 0.49	30.18 ± 2.61 ***	6.87 ± 0.56 ^^^	7.06 ± 0.50 ^^^
Tissue GSH (nmol/mg protein)	75.87 ± 4.68	75.38 ± 5.45	15.84 ± 3.76 ***	75.30 ± 6.90 ^^^	75.50 ± 4.81 ^^^
Tissue GSSG (nmol/mg protein)	7.27 ± 0.79	7.46 ± 1.16	34.14 ± 4.08 ***	7.49 ± 0.85 ^^^	7.67 ± 1.12 ^^^
Tissue GSH/GSSG	10.51 ± 1.08	10.29 ± 1.54	0.46 ± 0.12 ***	10.38 ± 1.55 ^^^	10.22 ± 1.42 ^^^
Tissue SOD (nmol/mg protein)	19.19 ± 1.48	20.83 ± 3.56	6.97 ± 0.41 ***	16.07 ± 3.22 ^^^	18.18 ± 2.46 ^^^
Tissue CAT (nmol/mg protein)	7.32 ± 0.38	7.67 ± 0.44	3.07 ± 0.46 ***	7.49 ± 0.58 ^^^	7.67 ± 0.53 ^^^
Tissue GR (nmol/mg protein)	34.84 ± 3.97	32.94 ± 5.19	9.60 ± 1.10 ***	29.45 ± 3.58 ^^^	30.19 ± 5.64 ^^^
Tissue GST (U/mg protein)	4.87 ± 0.29	4.24 ± 0.51	1.67 ± 0.30 ***	4.83 ± 0.32 ^^^	4.82 ± 0.72 ^^^
Tissue GPx (U/mg protein)	3.08 ± 0.30	3.28 ± 0.38	0.83 ± 0.30 ***	2.64 ± 0.22 ^^^	2.61 ± 0.31 ^^^
Tissue MPO activity (U/mg protein)	0.40 ± 0.07	0.43 ± 0.05	2.19 ± 0.25 ***	0.43 ± 0.12 ^^^	0.42 ± 0.12 ^^^
Tissue XO activity (nmol/mg protein)	0.53 ± 0.04	0.53 ± 0.04	1.65 ± 0.12 **	0.52 ± 0.07 ^^	0.50 ± 0.06 ^^
Tissue NO level (μM)	0.50 ± 0.06	0.44 ± 0.06	1.14 ± 0.10 **	0.57 ± 0.05 ^^	0.56 ± 0.08 ^^
Tissue NF-κB p65 level ng/mg protein	0.32 ± 0.03	0.35 ± 0.06	1.29 ± 0.12 **	0.35 ± 0.05 ^^	0.34 ± 0.04 ^^

Values are presented as mean ± SEM (***p< 0.001 vs. Sham; **p< 0.01 vs. Sham; ^^^p< 0.001 vs. IR; ^^p< 0.01 vs. IR)
CONP+IR (i.p.): Cerium oxide nanoparticle + ischemia reperfusion (intraperitoneally).
CONP+IR (o.g.): Cerium oxide nanoparticle + ischemia reperfusion (oral gavage).
LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MDA: Malondialdehyde, GSH: Reduced glutathione, GSSG: Oxidized glutathione, SOD: Superoxide dismutase, CAT: Catalase, GR: Glutathione reductase, GST: Glutathione S-transferase, GPx: Glutathione peroxidase, MPO: Myeloperoxidase, XO: Xanthine oxidase, NO: Nitric oxide, NF-κB: Nuclear factor kappa-B

Effects of CONPs on Oxidative Stress Parameters

Since oxidative stress is involved in hepatic ischemic damage, tissue oxidative stress parameters were estimated in control and study groups. Tissue level of MDA produced as a secondary product of lipid peroxidation was increased in the IR group compared to control and sham groups ($p< 0.001$). Treatment with CONPs decreased tissue lipid peroxidation levels significantly ($p< 0.001$) compared to those of the IR group. (Table 2). The administration of CONPs i.p. detected the most significant reduction in tissue MDA level, suggesting that CONPs, o.g. administration may be beneficial to the recovery of hepatic IR injury.

Liver GSH content was significantly decreased, GSSG content was increased, and GSH/GSSG ratio was decreased in the IR group compared to control and sham groups while GSH level and GSH/GSSG ratio were increased and GSSG level was decreased in the treatment groups ($p< 0.001$) (Table 2). Since tissue glutathione levels approached the levels of the control and sham groups with the treatment with CONPs, CONPs are presented as a promising therapeutic agent for the improvement of the impaired oxidative stress status during hepatic IR injury. The administration route of CONPs showed no statistical difference in terms of glutathione levels.

Tissue antioxidant enzyme activities were significantly decreased in liver tissues of the IR group and elevated in the treatment groups ($p< 0.001$). Thus, CONPs may be introduced as promising agents to recover the deteriorated antioxidant capacity during hepatic IR injury (Table 2). However, the administration route of CONPs did not show a statistical difference in antioxidant enzyme levels.

Table 3. Plasma proinflammatory cytokines and chemokines in the study groups

	Control	Sham	IR	CONP+IR (i.p.)	CONP+IR (o.g.)
TNF-α pg/mL	2.49 ± 0.49	2.41 ± 0.51	73.89 ± 5.12 ***	3.50 ± 0.80 ^^^	3.79 ± 1.24 ^^^
IL-1α pg/mL	4.76 ± 0.89	4.80 ± 1	17.21 ± 3.56 ***	5.82 ± 1.28 ^^^	5.60 ± 1.18 ^^^
IL-1β pg/mL	2.79 ± 0.48	3.02 ± 0.31	11.65 ± 1.69 ***	2.90 ± 1.30 ^^^	4.75 ± 0.86 ^^^
IL-2 pg/mL	18.01 ± 2.81	20.22 ± 3.94	67.66 ± 9.38 ***	24.17 ± 3.46 ^^^	27.49 ± 5.81 ^^^
IL-4 pg/mL	40.91 ± 3.14	42.34 ± 7.39	57.88 ± 11.07 *	45.91 ± 8.71 ^	41.79 ± 8.73 ^
IL-6 pg/mL	8.56 ± 0.82	8.14 ± 1.37	168.83 ± 21.24 ***	10.84 ± 1.55 ^^^	12.27 ± 2.55 ^^^
IL-8 pg/mL	112.37 ± 15.66	108.62 ± 16.17	503.34 ± 78.65 ***	124.52 ± 15.96 ^^^	144.46 ± 17.47 ^^^
IL-10 pg/mL	161.80 ± 16.38	172.63 ± 12.57	51.22 ± 1.07 ***	169.40 ± 7.66 ^^^	170.00 ± 6.80 ^^^
IL-12 pg/mL	116.41 ± 12.90	122.43 ± 18.31	1194.46 ± 129.99 ***	138.99 ± 24.66 ^^^	151.27 ± 32.20 ^^^
IL-17A pg/mL	47.67 ± 7.15	43.36 ± 7.94	435.46 ± 59.70 ***	50.98 ± 4.83 ^^^	55.22 ± 7.56 ^^^
ICAM-1 ng/mL	194.15 ± 2.62	194.39 ± 4.82	551.56 ± 77.01 ***	202.22 ± 6.15 ^^^	208.02 ± 6.12 ^^^
MMP-2 pg/mL	71.05 ± 14.23	71.04 ± 12.45	244.62 ± 147.25 ***	74.57 ± 16.64 ^^^	85.23 ± 11.60 ^^^
MMP-9 pg/mL	63.58 ± 2.90	66.21 ± 3.00	562.03 ± 16.03 ***	100.99 ± 23.08 ^^^	116.17 ± 18.07 ^^^
TIMP-1 pg/mL	57.25 ± 1.45	56.34 ± 1.90	12.30 ± 2.00 ***	58.08 ± 2.20 ^^^	60.11 ± 3.55 ^^^

Data are presented as mean ± SEM (***$p< 0.001$ vs. sham; *$p< 0.05$ vs. sham; ^^^$p< 0.001$ vs. IR; ^$p< 0.05$ vs. IR).
TNF- α: Tumor necrosis factor, IL: Interleukin, ICAM-1: Intercellular adhesion molecule, MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase.

Effects of CONPs on Tissue MPO Activity

MPO activity, a biomarker to indicate the degree of neutrophil accumulation and inflammatory response, was elevated in the IR group compared to control and sham groups ($p< 0.001$). Conversely, CONP administration significantly decreased hepatic MPO activity than the IR group ($p< 0.001$) (Table 2).

Effects of CONPs on Tissue XO Activity

XO activity in liver tissue was significantly increased in the IR group in comparison with the control and sham groups. On the other hand, CONP administration significantly suppressed the hepatic XO activity ($p< 0.01$) (Table 2).

Effects of CONPs on Tissue NF-κB Activity

NF-κB activity in liver tissue was found to be dramatically increased in the IR group, whereas this level was importantly increased in CONP treatment groups ($p< 0.01$) (Table 2). The administration route of CONPs did not show a statistical difference in hepatic NF-κB levels.

Effects of CONPs on Plasma Cytokine Levels

IR injury causes a complex inflammatory immune response and is associated with a marked increase in inflammatory mediators and chemotactic proteins (11). In accordance with the previous studies, it was observed that IR injury significantly altered serum inflammatory proteins (Table 3). Plasma TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-17A, ICAM-1 levels were markedly increased, and IL-10 was decreased in the IR group while pretreatment with CONPs approached the altered cytokine/chemokine/adhesion molecule levels of control and sham groups ($p< 0.001$) (Table 3). In addition, the tissue expression of TNF-α was increased in IR group and decreased in the treatment groups ($p< 0.001$) (Figure 4), indicating that CONP administration reduces the enhanced TNF-α production.

Effects of CONPs on Plasma MMP Levels

Since hepatic IR is associated with MMP activation and release, plasma MMP-2, MMP-9, and TIMP-1 levels were measured in control and study groups. Plasma MMP-2 and MMP-9 levels were significantly higher in IR group compared to the values of control and sham groups ($p< 0.001$). Conversely, MMP expression and plasma levels were markedly decreased in the CONPs administration groups ($p< 0.001$). (Figure 5).

DISCUSSION

Hepatic IR injury represents a clinical problem associated with many surgical interventions. The induction of Kupffer cell after reperfusion is the initial phase of liver and followed by ROS release, which generates oxidative stress, parenchymal and vascular injuries, as well as hepatocyte damage via lipid peroxidation or straightly enhancing neutrophil microcirculation (12). Cytokines released by activated Kupffer cells and aggregated neutrophils play a crucial role in IR injury (13).

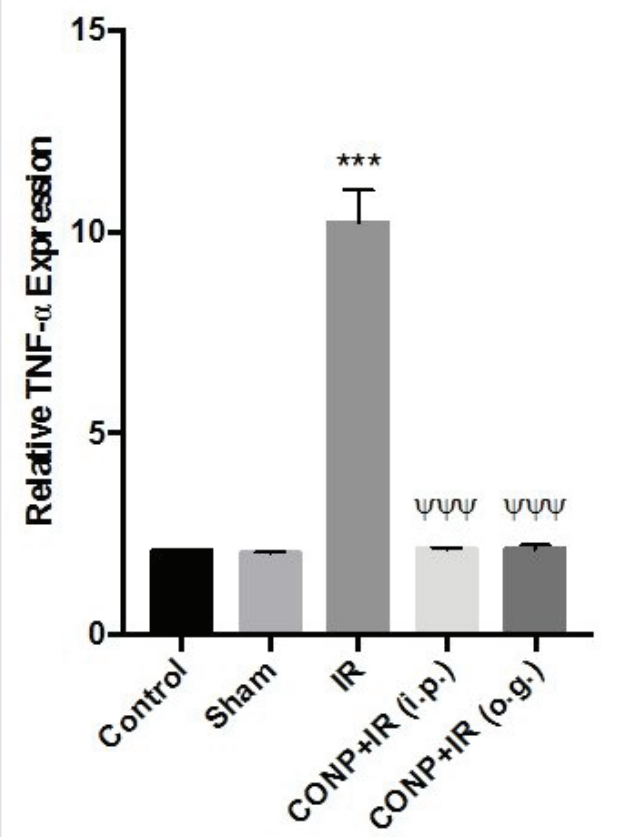

Figure 4. Gene expression of TNF-α in liver tissue of study groups. Values are presented as mean ± SEM (n= 6). (***$p< 0.001$ vs Sham; ΨΨΨ$p< 0.001$ vs IR).

CONPs have shown to display several antioxidant behaviors, including SOD activity, CATmimetic activity, NO radical scavenging, hydroxyl radical scavenging, radiation-protective anti-inflammatory, and neuroprotective effects (14). It has been reported that CONPs might be used to treat sepsis, cardiomyopathy, stroke, obesity, ovarian cancer, hepatic steatosis, and cancer (7,15-18). Previous studies have suggested that CONPs show SOD-like, CAT-like, and peroxidase activity and NO scavenging ability, leading to reduced ROS production in macrophages (19).

Treatment of hepatic IR injury with antioxidants has exhibited encouraging results in vivo but has not been successful in clinical applications resulting from insufficient antioxidant levels. Thus, targeted drug delivery would provide better outcomes. The studies have demonstrated that upon i.v. injection, before translocating to the organs, CONPs stay in circulation for a short period (t1/2 is 7.5 min) (9). The Kupffer cells enclose CONPs within the liver. Since reperfusion is followed by a dramatically increased oxidative stress, administering CONPs 1 h before ischemia results in bioaccumulation of the particles in the liver that scavenge ROS that are formed during reperfusion (20). In the present study, hepatic IR caused a dramatic elevation in

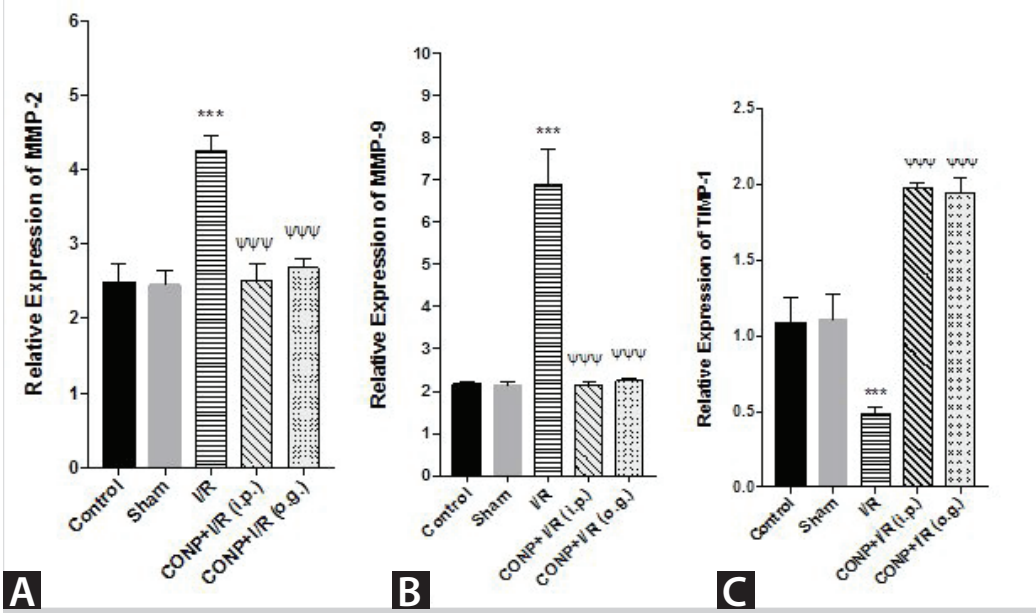

Figure 5. Gene expressions of MMP-2 **(A)** MMP-9 **(B)** and TIMP-1 **(C)** in liver samples of study groups. Values are presented as mean ± SEM (n= 6). (***p< 0.001 vs Sham; ΨΨΨp< 0.001 vs IR).

plasma ALT, AST, and LDH enzymes due to IR-induced hepatic cell injury. Treatment with CONPs 24 hours before the IR in the liver attenuated increases in plasma ALT-AST and LDH activities, indicating that CONP administration has the benefit of reducing hepatocellular injury during IR. CONP administration also reduced the elevated levels of MDA, GSSG while increasing the levels of GSH and antioxidant enzymes. Severe hepatocyte swelling, alongside vacuolar degeneration and multiple necrotic areas, are common findings during the histological analysis of the tissues with IR injury. Conversely, treatment with CONPs recovered these changes supporting the findings that CONPs in oxidized form transits between cerium (III) oxide (Ce^{3+}) and Ce^{4+} oxidative states, allow regenerative redox cycling and free radical scavenging (21).

During IR injury, NO production is compromised because of constitutive endothelial NO synthase dysfunction. It has been shown that NO reduces macrophage and neutrophil infiltration and neutralizes the superoxide anion. It also inhibits apoptosis, protects the sinus structure of liver and microcirculatory blood flow, increases hepatic oxygenation, and diminishes oxidative stress injury (1). In our study, tissue NO level was increased in the hepatic IR group and decreased in CONP administration groups, possibly due to the antioxidative properties of CONPs.

XO, a rate-limiting enzyme of purine catabolism, operates as a ROS source in IR injury (22). Under ischemic conditions, XO occurs with proteolysis from xanthine dehydrogenase (XDH). In normoxic conditions, XDH produces urate from hypoxanthine and xanthine, and XO, whose expression is the highest in the liver, is responsible for the ROS generation under hypoxic and IR conditions (23). Our data confirmed that CONP administration reduced the plasma levels of XO and ICAM-1 and ameliorated the cellular liver damage during hepatic IR.

It has been demonstrated that NF-κB activation endorses the levels of cytokines such as TNF-α, and IL-6, in the initial phase of the injury in the Kupffer cells (12). In this study, tissue NF-κB level was elevated in the IR group and reduced in CONP administration groups, demonstrating that CONP administration has a beneficial effect on regulating NF-κB levels and oxidative unbalance in hepatic IR.

Studies have indicated that MMPs and their TIMPs play significant roles in the extracellular matrix remodeling in liver damage (24). Proteases are delivered from injured cells, when healthy cells deliver TIMPs. A high ratio of MMP/TIMP indicates the activated MMPs, whereas a low ratio of MMP/TIMP hints at the contrary. MMP-2 and MMP-9, significantly ensured in the degradation of fibronectin and collagen IV, may cause damage in the liver to altering the sinusoidal cells and remodeling of the stromal structure (25). The tissue expressions and plasma levels of MMP-2 and -9 were increased while tissue expression and plasma level of TIMP-1 decreased in the IR group, demonstrating a high MMP/TIMP ratio hepatic IR injury in this study. Since CONP administration reversed this unbalanced status, it was concluded that CONPs administration prevents the activation of matrix proteinases and protects the liver from IR injury.

Hepatic IR injury causes an enhancement in proinflammatory mediators and chemotactic proteins (9). In accordance with previous studies, we found that hepatic IR injury led to increased plasma inflammatory proteins such as plasma TNF-α,

IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-17A, ICAM-1 levels and decreased plasma IL-10 level compared to control and sham groups. However, the changes in inflammatory markers are diminished and returned almost to the baseline controls via treatment with CONPs. Additionally, TNF-α increases the expression of adhesion molecules like ICAM-1, vascular cell adhesion protein (VCAM-1), and P-selectin on vascular endothelial cells (6). Reducing tissue TNF-α expression and plasma TNF-α level is suggested to improve hepatic IR injury by suppressing inflammatory response (26).

CONCLUSION

CONPs scavenge reactive oxygen and nitrogen species by altering the enzymes in favor of antioxidation or non-enzymatic ways through scavenging hydroxyl and NO radicals. Most experimental studies related to CONPs are principally focused on animal or human cells in vitro; thus, more in vivo studies are needed.

This preliminary study demonstrates a significant reduction in liver degeneration by administering CONPs via i.p. and o.p. route in an experimental liver IR model. Presented data suggest that CONPs have the potential for the prevention of hepatic IR injury. However, the details of the mechanism of the cytoprotective effect produced by CONPs should be further investigated. Furthermore, cytokine and MMP levels examined at the protein level should also be examined at the gene level to determine whether the effect is at the gene level or the protein level, so the details of the mechanism should be clarified.

Main Points

- Hepatic ischemia-reperfusion (IR) injury is a complication of liver-related surgical interventions.
- Cerium oxide nanoparticles (CONPs) are used to treat oxidative stress and inflammation-related processes.
- Preoperative treatment with CONPs reduces the increased oxidative stress, pro-inflammatory mediators, and extracellular matrix components in hepatic IR.
- CONPs may be considered as promising therapeutic agents for preventing IR injury.

Ethics Committee Approval: The approval for this study was obtained from Dumlupınar University Animal Experiments Local Ethics Committee with the Decision no: 2017.04-02, Date: 06.04.2017).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.Z., A.E., G.T.; Design – A.Z., A.E., G.T.; Supervision – G.U., M.C.A., B.G.; Materials – A.Z., A.E., G.T.; Data Collection and/ or Processing – K.K., G.T.; Analysis and/or Interpretation – G.U., M.C.A., B.G.; Literature Search – A.Z., K.K.; Writing Manuscript – A.Z., G.U., K.K.; Critical Reviews – G.U., M.C.A., B.G.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. *Guan LY, Fu PY, Li PD, Li ZN, Liu HY, Xin MG, et al. Mechanisms of hepatic ischemia-reperfusion injury and protective effects of nitric oxide. World J Gastrointest Surg 2014; 6(7): 122-8. https://doi.org/10.4240/wjgs.v6.i7.122*
2. *Wu M, Yiang G, Liao WT, Tsai APY, Cheng YL, Cheng PW, et al. Current mechanistic concepts in ischemia and reperfusion injury. Cell Physiol Biochem 2018; 46: 1650-67. https://doi.org/10.1159/000489241*
3. *Klune JR, Tsung A. Molecular biology of liver ischemia/reperfusion injury: Established mechanisms and recent advancements. Surg Clin 2010; 90: 665-77. https://doi.org/10.1016/j.suc.2010.04.003*
4. *Liu A, Huang L, Fan H, Fang H, Yang Y, Liu S, et al. Baicalein pretreatment protects against liver ischemia/reperfusion injury via inhibition of NF-κB pathway in mice. Int Immunopharmacol 2015; 24: 72-9. https://doi.org/10.1016/j.intimp.2014.11.014*
5. *Ferreira CA, Ni D, Rosenkrans ZT, Cai W. Scavenging of reactive oxygen and nitrogen species with nanomaterials. Nano Res 2018; 11: 4955-84. https://doi.org/10.1007/s12274-018-2092-y*
6. *Nelson BC, Johnson ME, Walker ML, Riley KR, Sims CM. Antioxidant cerium oxide nanoparticles in biology and medicine. Antioxidants 2016; 5(2): 15-36. https://doi.org/10.3390/antiox5020015*
7. *Estevez AY, Pritchard S, Harper K, Aston JW, Lynch A, Lucky JJ, et al. Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia. Free Radic Biol Med 2011; 51: 1155-63. https://doi.org/10.1016/j.freeradbiomed.2011.06.006*
8. *Ribera J, Rodríguez-Vita J, Cordoba B, Portolés I, Casals G, Casals E, et al. Functionalized cerium oxide nanoparticles mitigate the oxidative stress and pro-inflammatory activity associated to the portal vein endothelium of cirrhotic rats. PloS One 2019; 14:e0218716. https://doi.org/10.1371/journal.pone.0218716*
9. *Manne ND, Arvapalli R, Graffeo VA, Bandarupalli VV, Shokuhfar T, Patel S, et al. Prophylactic treatment with cerium oxide nanoparticles attenuate hepatic ischemia reperfusion injury in sprague-dawley rats. Cell Physiol Biochem 2017; 42: 1837-46. https://doi.org/10.1159/000479540*
10. *Kleiner DE. Drug-induced liver injury: The hepatic pathologist's approach. Gastroenterol Clin 2017; 46:273-96. https://doi.org/10.1016/j.gtc.2017.01.004*
11. *Adebayo OA, Akinloye O, Adaramoye OA. Cerium oxide nanoparticles attenuate oxidative stress and inflammation in the liver of diethylnitrosamine-treated mice. Biol Trace Elem Res 2020; 193: 214-25. https://doi.org/10.1007/s12011-019-01696-5*
12. *Konishi T, Lentsch AB. Hepatic ischemia/reperfusion: mechanisms of tissue injury, repair, and regeneration. Gene Expr J Liver Res 2017; 17: 277-87. https://doi.org/10.3727/105221617X15042750874156*
13. *Peralta C, Jiménez-Castro MB, Gracia-Sancho J. Hepatic ischemia and reperfusion injury: effects on the liver sinusoidal milieu. J Hepatol 2013; 59: 1094-106. https://doi.org/10.1016/j.jhep.2013.06.017*
14. *Wason MS, Zhao J. Cerium oxide nanoparticles: Potential applications for cancer and other diseases. Am J Transl Res 2013; 5(2): 126-31.*
15. *Niu J, Wang K, Kolattukudy PE. Cerium oxide nanoparticles inhibits oxidative stress and nuclear factor-κB activation in H9c2 cardiomyocytes exposed to cigarette smoke extract. J Pharmacol Exp Ther 2011; 338: 53-61. https://doi.org/10.1124/jpet.111.179978*
16. *Wasef L, Nassar AM, El-Sayed YS, Samak D, Noreldin A, Elshony N, et al. The potential ameliorative impacts of cerium oxide nanoparticles against fipronil-induced hepatic steatosis. Sci Rep 2021; 11: 1-15. https://doi.org/10.1038/s41598-020-79479-5*

17. *Corsi F, Caputo F, Traversa E, Ghibelli L. Not only redox: the multifaceted activity of cerium oxide nanoparticles in cancer prevention and therapy. Front Oncol 2018; 8: 309-15. https://doi.org/10.3389/fonc.2018.00309*

18. *Tuncay A, Sivgin V, Ozdemirkan A, Sezen SC, Boyunaga H, Kucuk A, et al. The effect of cerium oxide on lung tissue in lower extremity ischemia reperfusion injury in sevoflurane administered rats. Int J Nanomedicine 2020; 15: 7481-9. https://doi.org/10.2147/IJN.S263001*

19. *Casals E, Zeng M, Parra-Robert M, Fernández-Varo G, Morales-Ruiz M, Jiménez W, et al. Cerium oxide nanoparticles: Advances in biodistribution, toxicity, and preclinical exploration. Small 2020; 16: 1907322-43. https://doi.org/10.1002/smll.201907322*

20. *Xu Z, Yu J, Wu J, Qi F, Wang H, Wang Z, et al. The effects of two anesthetics, propofol and sevoflurane, on liver ischemia/reperfusion injury. Cell Physiol Biochem 2016; 38: 1631-42. https://doi.org/10.1159/000443103*

21. *Selvaraj V, Nepal N, Rogers S, Manne ND, Arvapalli R, Rice KM, et al. Inhibition of MAP kinase/NF-kB mediated signaling and attenuation of lipopolysaccharide induced severe sepsis by cerium oxide nanoparticles. Biomaterials 2015; 59: 160-71. https://doi.org/10.1016/j.biomaterials.2015.04.025*

22. *Choi EK, Jung H, Kim K-J, Kang SJ, Kim HJ, Lim JA, et al. Sodium nitrite attenuates hepatic ischemia-reperfusion injury in rats. Exp Clin Transplant Off J Middle East Soc Organ Transplant 2018; 17: 348-54. https://doi.org/10.6002/ect.2018.0169*

23. *Tanno S, Yamamoto K, Kurata Y, Adachi M, Inoue Y, Otani N, et al. Protective effects of topiroxostat on an ischemia-reperfusion model of rat hearts. Circ J 2018; 82: 1101-11. https://doi.org/10.1253/circj.CJ-17-1049*

24. *Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol 2018; 68: 452-62. https://doi.org/10.1016/j.matbio.2017.11.011*

25. *Palladini G, Ferrigno A, Richelmi P, Perlini S, Vairetti M. Role of matrix metalloproteinases in cholestasis and hepatic ischemia/reperfusion injury: A review. World J Gastroenterol 2015; 21(42): 12114-24. https://doi.org/10.3748/wjg.v21.i42.12114*

26. *Inbaraj BS, Chen B-H. An overview on recent in vivo biological application of cerium oxide nanoparticles. Asian J Pharm Sci 2020; 15: 558-75. https://doi.org/10.1016/j.ajps.2019.10.005*

ORİJİNAL ÇALIŞMA-ÖZET
Turk J Surg 2022; 38 (3): 255-265

Deneysel karaciğer iskemi-reperfüzyon hasarında cerium oksidin oral ve intraperitoneal uygulanmasının anti-enflamatuvar etkisi

Akile Zengin[1], Açelya Erikçi[2], Gökçen Telli[3], Bülent Gümüşel[4], Kemal Kösemehmetoğlu[5], Gülberk Uçar[6], Mustafa Cem Algın[7]

1 Malatya Eğitim ve Araştırma Hastanesi, Gastrointestinal Cerrahi Kliniği, Malatya, Türkiye
[2] Lokman Hekim Üniversitesi Eczacılık Fakültesi, Biyokimya Anabilim Dalı, Ankara, Türkiye
[3] Hacettepe Üniversitesi Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, Ankara, Türkiye
[4] Lokman Hekim Üniversitesi Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, Ankara, Türkiye
[5] Hacettepe Üniversitesi Tıp Fakültesi, Patoloji Anabilim Dalı, Ankara, Türkiye
[6] Hacettepe Üniversitesi Eczacılık Fakültesi, Biyokimya Anabilim Dalı, Ankara, Türkiye
[7] Kütahya Sağlık Bilimleri Üniversitesi, Genel Cerrahi Anabilim Dalı, Kütahya, Türkiye

ÖZET

Giriş ve Amaç: Hepatik iskemi-reperfüzyon (IR) hasarı karaciğer cerrahisi ve transplantasyonda meydana gelir. IR hücre hasarı kaskadını, nekroz/ apopitoz ve hepatosellüler hasarı yöneten intrasellüler sinyal kaskadını aktive olmasıyla oluşan proenflamatuvar cevaplar ile reaktif oksijen radikalleri (ROS) üretilir. Cerium oksit nanopartikülleri (CONPs) anti-enflamatuvar ve antioksidan ajan gibi davranmaktadır. Bu yüzden, CONPs'nin oral (o.g.) ve intraperitoneal (i.p.) uygulanmasının hepatic IR hasarındaki koruyucu etkisini değerlendirdik.

Gereç ve Yöntem: Fareler rastgele kontrol, sham, IR protocol, CONP+ IR (i.p.) ve CONP (o.g.) olarak beş gruba ayrıldı. Fare hepatik IR protokolü IR grubundaki hayvanlara uygulandı. IR protokolünden 24 saat önce CONPs (300 µg/kg) uygulandı. Reperfüzyon periyodu sonrası kan ve doku örnekleri alındı.

Bulgular: Hepatik IR hasarı, doku lipid peroksidasyonunu, miyeloperoksidaz (MPO), ksantin oksidaz (XO), nitrit oksit (NO) ve doku nükleer faktör kappa-B(NF-κB) p65 enzim aktivitelerinin seviyelerini belirgin şekilde arttırdı; plazma proenflamatuvar sitokinler, kemokinler ve adezyon molekülleri antioksidan belirteçleri azaltırken karaciğer dokusunda patolojik değişikliklere neden olmuştur. IR grubunda tümör nekroz faktör alfa (TNF-α), matriks metalloproteinaz 2 (MMP-2) ve 9 ekspresyonu artmış, doku inhibitörü matriks metalloproteinaz 1 (TIMP-1) ekspresyonu azalmıştır. Hepatik iskemiden 24 saat önce o.g. ve i.p. olarak uygulanan CONP'ler ile yukarıda belirtilen biyokimyasal parametreleri düzeldi ve histopatolojik özellikleri hafifletti.

Sonuç: Bu çalışmanın sonuçları, deneysel bir karaciğer IR modelinde i.p. ve o.g. yoluyla CONP'lerin uygulanmasıyla karaciğer dejenerasyonunda önemli bir azalma olduğunu göstermektedir, bu da CONP'lerin hepatik IR hasarının önlenmesi için geniş bir potansiyele sahip olduğunu göstermektedir.

Anahtar Kelimeler: İskemi reperfüzyon, cerium oksit, nanopartiküller (CONPs), fare, oksidatif stres, enflamasyon

DOİ: 10.47717/turkjsurg.2022.5620