



Impact of garlic (*Allium sativum*) oil on cisplatin-induced hepatorenal biochemical and histopathological alterations in rats

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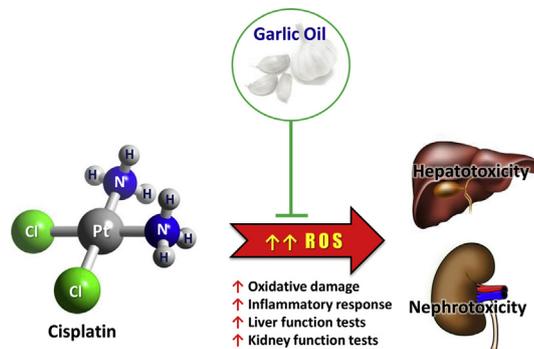
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HIGHLIGHTS

- Cisplatin induced hepatorenal oxidative damages in rats.
- Cisplatin provoked hepatic and renal tissue lesions.
- Garlic oil has antioxidant and anti-inflammatory actions.
- Garlic oil gets over cisplatin adverse effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Cisplatin (cis-diamminedichloridoplatinum II [CDDP]) is a chemotherapeutic agent used for treating different cancers types. However, its usage is limited because it induces harmful toxicities in multiple organs, including nephrotoxicity and hepatotoxicity. Garlic oil (GO) has several pharmacological activities, including antioxidant activity. The aim of the study is to evaluate the protective and antioxidant effects of GO against CDDP-induced acute liver and kidney injuries in male rats. CDDP-treated rats showed increased serum ALT, AST, ALP, LDH, uric acid, urea, creatinine, and IL-6 levels. Moreover, CDDP-treated rats showed significantly increased MDA and NO levels and decreased GSH level and T.SOD and CAT activities in hepatic and renal tissues compared with control rats. GO administration, especially at a dose of 100 ml/kg, alleviated CDDP-induced adverse

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CDDP, cis-diamminedichloridoplatinum II; DADS, diallyl disulfide; GSH, reduced glutathione; LDH, lactate dehydrogenase; MDA, malondialdehyde; NO, nitric oxide; PAS, periodic acid-Schiff; ROS, reactive oxygen species; SOD, superoxide dismutase.

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biochemical and histopathological alterations and restored them to their normal values. These results suggest that GO reverses CDDP-induced hepatorenal damage by exerting antioxidant and anti-inflammatory effects.

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1. Introduction

Cisplatin or cis-diamminedichloridoplatinum II (CDDP) is a drug used for treating solid tumors such as head, neck, ovarian, bladder, breast, lung, and cervical cancers (Galanski, 2006). Renal toxicity is the main concern associated with the clinical use of CDDP (Volarevic et al., 2019). Therefore, researchers are assessing strategies to control CDDP-induced oxidative damage while maintaining its anticarcinogenic activity. CDDP is metabolized to a potent nephrotoxin (Yao et al., 2007). Indeed, unbound plasma CDDP is filtered by the glomerulus and most of the CDDP is deposited in the renal cortex leading to nephrotoxicity (Wei et al., 2007). Moreover, CDDP can induce hepatotoxicity when administered at high doses (Niu et al., 2017; Sudhakar et al., 2010). CDDP decreases the levels of antioxidant enzymes, leading to the cellular accumulation of reactive oxygen species (ROS) and lipid peroxidation products that induce oxidative stress and pathogenic diseases (Sadzuka et al., 1992). ROS decrease renal blood flow and induce tubular damage (Matsushima et al., 1998; Soni et al., 2018). Moreover, ROS induce cell necrosis by promoting cell membrane lipid peroxidation, protein alkylation, and DNA damage (Atessahin et al., 2005). CDDP conjugates with vital molecules such as glutathione, proteins, RNA, and DNA (Siddik, 2003). CDDP cytotoxicity correlates with DNA adduct formation that blocking the DNA replication, encourage a G2 cell cycle arrest and hinder RNA transcription and lastly promote cell death (Garrido et al., 2008).

Oxidative stress can be prevented or alleviated by using antioxidants that scavenge ROS of external and internal origin. Superoxide dismutase (SOD, EC 1.15.1.1) is a potent endogenous antioxidant enzyme that converts superoxide anions into H₂O₂. Hence, the decline in the oxidative damage (Muller et al., 2006). Another cellular antioxidant enzyme catalase (CAT, E.C. 1.11.1.6) protects cells from ROS-induced oxidative damage by converting H₂O₂ to H₂O and O₂ (Kim et al., 2009).

Garlic (*Allium sativum*) has been used as a traditional plant for many years and has been reported to exhibit anticarcinogenic, anti-atherosclerotic, antidiabetic, renoprotective, antioxidant, anti-inflammatory, immune and antibacterial properties (Capasso, 2013; Chauhan and Mehla, 2015; Jangam and Badole, 2014). Garlic oil (GO) is a commercially available garlic product which contains the major bioactive constituents of garlic including organosulfur substances such as diallyl sulfide, diallyl disulfide (DADS), diallyl trisulfide, and allyl methyl trisulfide (Abdel-Daim et al., 2015; Abdel-Daim and Abdou, 2015; Abdel-Daim et al., 2017; Block, 1992). Garlic exerts antioxidant effects by scavenging free radicals and by conserving cellular integrity (Khanum et al., 2004).

The clinical use of cisplatin as a potent anticancer drug is often limited due to undesirable side effects such as nephrotoxicity, and more recently investigators have demonstrated hepatotoxicity induced by oxidative stress. While nephrotoxicity of cisplatin is common and well documented, hepatotoxicity received less attention. We thus focused on cisplatin-induced renal and hepatic toxicity along with investigating the potential use of garlic oil as a protective agent to overcome these toxicities. To achieve such an objective, renal and hepatic serum biomarkers and tissue oxidative indices in rats were assessed over 10 days along with performing histopathological and histochemical analyses.

2. Materials and methods

2.1. Chemicals

Cisplatin (commercially available as Cisplatin MYLAN® [1 mg/ml]) was obtained from Mylan Pharmaceuticals, France. GO was obtained from El Captain Company (Cairo, Egypt). All analytical kits were obtained from Biodiagnostic Company (Cairo, Egypt), except IL-6, which was purchased from Glory Science Co., Ltd. (Del Rio, TX, USA).

2.2. Gas chromatography–mass spectrometry analysis

Garlic oil was dissolved in *n*-hexane to obtain a dilution of 1:3 (*v*:*v*). Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a method described by Guo et al. (2005), with a splitless mode. Briefly, 10 µl GO–*n*-hexane mixture was injected in Trace GC Ultra-ISQ mass spectrometer with a direct capillary column (TG–5MS, 30 m × 0.25 mm × 0.25 µm). Column temperature was maintained at 80 °C–300 °C at a rate of 5 °C/min and 300 °C for 20 min. Injector temperature was 270 °C, detector temperature was 230 °C, and carrier gas (helium) flow rate was 1.5 m/min. Mass spectra of identified components were determined by comparing with Wiley Registry of Mass Spectral Database, 8th Edition.

2.3. Animals and experimental design

The present study included 32 male Wister rats (weight, 150 ± 15 g) that were obtained from Laboratory Animal House of Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. The study was performed according to the institutional animal care guidelines of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

The animals were housed under standard laboratory conditions of standard room temperature (22 °C ± 3 °C), constant humidity, and 12-/12-h light/dark cycle. The rats were given *ad libitum* access to water and food. Ingredients of experimental diet given to the rats are listed in Table S1.

The rats were acclimatized to the laboratory conditions one week before performing the experiment. Next, the rats were randomly divided into the following four groups, with eight rats per group: (1) rats intraperitoneally (i.p.) injected with normal saline (control group), (2) rats i.p. injected with a single 5 mg/kg dose of CDDP on day 6 of the experiment (Mashhadi et al., 2014) (CDDP group), (3) rats treated orally with 50 ml/kg GO using gastric gavage for 10 days and i.p. injected with 5 mg/kg CDDP on day 6 of the experiment (CDDP-GO₅₀) group, and (4) rats treated orally with 100 ml/kg GO using gastric gavage for 10 days and i.p. injected with 5 mg/kg CDDP on day 6 of the experiment (CDDP-GO₁₀₀) group. The doses of GO were selected according to the previous literature (Liu et al., 2012). Blood and tissue samples were collected from animals in all the groups on day 11.

2.4. Serum collection and tissue sampling

At the end of the experiment, blood was collected from the rats by performing direct heart puncture. The blood was placed in plain tubes, left at room temperature to promote clot formation, and centrifuged

at 3000 rpm for 15 min. The obtained clear sera were used for performing biochemical analysis.

After blood collection, the rats were sacrificed, and their liver and kidneys were removed immediately. Tissues surrounding the isolated organs were removed, and the organs were washed with sodium chloride solution (0.9%). Next, the tissues were dried using a filter paper and were immersed in ice-cold phosphate-buffered saline containing 0.1 mM EDTA to remove coagulated blood. Next, the isolated liver and kidney tissues were divided into two parts. One part was stored at -80°C for preparing tissue homogenates to assess malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) levels and to spectrophotometrically analyze T.SOD and CAT activities. The other part was stored in 10% neutral-buffered formalin for performing histopathological analysis.

2.5. Serum biochemical analysis

The activities of aspartate aminotransferase (AST, EC 2.6.1.1) (Reitman and Frankel, 1957), alanine aminotransferase (ALT, EC 2.6.1.2) (Reitman and Frankel, 1957), alkaline phosphatase (ALP, EC 3.1.3.1) (Tietz et al., 1983), and lactate dehydrogenase (LDH, EC 1.1.1.27) (Zimmerman and Henery, 1979) were determined in collected sera as markers for liver injury. In addition, the serum levels of uric acid (Whitehead et al., 1991), urea (Coulombe and Favreau, 1963), and creatinine (Bartels et al., 1972) were determined to evaluate kidney function.

2.6. Determination of tissue oxidative stress and antioxidant status

2.6.1. Preparation of tissue homogenates

At the end of the study, the rats ($n = 8$) from each group were sacrificed under anesthesia by intramuscularly injecting sodium pentobarbital (50 mg/kg BW). Their liver and kidneys were removed immediately and were immersed in ice-cold saline (0.9%). The isolated organs were homogenized using a motor-driven Teflon and glass Potter-Elvehjem homogenizer in 0.1 M Tris-HCl buffer (pH 7.4) containing 5 mM β -mercaptoethanol (1:4 w/v). The tissue homogenates were centrifuged at 105,000 $\times g$ and 4°C for 60 min. Supernatants obtained were divided into aliquots and were stored at -80°C for evaluating oxidative stress and antioxidant status.

2.6.2. Determination of MDA level

The level of MDA was estimated by incubating the supernatants with thiobarbituric acid (pH 3.6) at 95°C for 30 min to form a pink-colored thiobarbituric acid reactive substance. MDA level was determined by measuring absorbance at 532 nm, and is expressed as nmol MDA/g tissue (Ohkawa et al., 1979).

2.6.3. Determination of NO level

In the acid medium and in the presence of nitrite, the formed nitrous acid that reacts with sulphanilamide and the product is united with *N*-(1-naphthyl) ethylenediamine resulting in an azo dye of a bright reddish – purple color that was colorimetrically detected at 540 nm, and expressed as $\mu\text{mol/g}$ tissue (Montgomery and Dymock, 1961).

2.6.4. Determination of GSH level

The assay for determining GSH level depends on the reductive cleavage of 5,5'-dithiobis (2-nitrobenzoic acid) by compounds containing sulfhydryl groups that produce yellow-colored products (Sedlak and Lindsay, 1968). The decrease in the amount of chromogen corresponds to the GSH level. GSH level was determined by measuring absorbance at 412 nm and is expressed as $\mu\text{mol GSH/g}$ tissue.

2.6.5. Determination of T.SOD activity

The assay for determining T.SOD activity depends on the ability of SOD to repress phenazine methosulfate-induced reduction of nitro

blue tetrazolium through NADH-mediated under aerobic conditions. T. SOD activity was determined by measuring absorbance at 560 nm, and is expressed as U/g tissue (Nishikimi et al., 1972).

2.6.6. Determination of CAT activity

Catalase is activated in the presence of distinguishable levels of H_2O_2 . In the present study, CAT activity was arrested for 1 min by using a CAT inhibitor. In the presence of horseradish peroxidase, residual H_2O_2 reacted with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to produce a chromophore whose color intensity was inversely proportional to CAT level in the original sample. CAT activity was measured based on an increase in absorbance at 560 nm for 5 min, with 1-min interval and expressed as U/g tissue (Aebi, 1984).

2.7. ELISA

Serum IL-6 level was determined by performing ELISA (Glory Science Co., TX, USA), according to the manufacturer's instructions, and expressed as pg/ml.

2.8. Histopathological analysis

The liver and kidney tissues were removed immediately after sacrificing the rats, fixed in 10% neutral-buffered formalin, dehydrated using gradual ethanol concentrations (50%–100%), and embedded in paraffin after treatment with xylene. The paraffin-embedded tissues were sectioned into 4- to 5- μm -thick sections and were stained with hematoxylin and eosin (H&E) (Bancroft et al., 1996).

2.9. Histochemical analysis

For performing semi-quantitative analysis and for determining lesion scores, the paraffin-embedded kidney and liver tissues were sectioned into 5- μm -thick sections and were stained with periodic acid-Schiff (PAS) (Bancroft et al., 1996).

Lesions in the liver tissue were evaluated and scored by performing semi-quantitative analysis and by determining PAS staining intensity of positively stained regions as measure of cellular activity after subtracting background noise. PAS staining intensity was determined using ImageJ software (National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij/>). Ten fields were randomly selected from each slide for all the experimental groups, and integrated density (IntDen) of the 10 random fields was determined. Mean IntDen is expressed as field IntDen.

Lesions in the renal tissue were evaluated and scored as described previously (Ramesh et al., 2007). Acute tubular necrosis was assessed in the outer strip of the medulla and cortex using a semi-quantitative scale in which the percentage of tubules showing epithelial necrosis, brush-border loss, and cast formation was assigned a score: 0 = normal; 1 \leq 10%; 2 = 11–25%; 3 = 26–45%; 4 = 46–75%; 5 \geq 76%. Renal lesions in 10 randomly selected fields were examined and averaged. The tissue sections were scored in a blinded manner.

2.10. Statistical analysis

All data were statistically analyzed using one-way ANOVA with Duncan's multiple comparison test by using SPSS software program, version 20.0 (SPSS Inc., Chicago, IL). A P value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. GC–MS analysis

The chemical composition of GO, as determined by performing the GC–MS analysis, is shown in Table S2. Results of the GC–MS analysis showed that the percentages of DADS, 2,2-dideutero-octadecanal, oleic acid, 1,54-dibromo tetrapentacontane, dotriacontane, and isochiapiin B in GO were 0.60%, 3.03%, 2.58%, 4.80%, 10.99%, and 2.61%, respectively. Hence, GO used in the present study has been referenced as 0.60% equivalent of DADS.

3.2. Serum biochemical analysis

Cisplatin (5 mg/kg BW) injection on day 6 of the experiment significantly increased serum ALT, AST, ALP, and LDH activities compared with those in control rats. Similarly, CDDP (5 mg/kg BW) injection significantly increased the levels of renal injury biochemical markers. In addition, serum IL-6 level was significantly increased due to CDDP injection compared to that in control rats ($P \leq 0.05$; Table 1).

Rats in the CDDP-GO₅₀ and CDDP-GO₁₀₀ groups showed significantly decreased levels of renal biochemical markers (uric acid, urea, and creatinine; $P \leq 0.05$) compared with rats in the CDDP group. In rats in the CDDP-GO₁₀₀ group, 100 ml/kg GO administration completely restored the serum levels of all the tested enzymes and biomarkers to their normal values. In rats in the CDDP-GO₅₀ group, 50 ml/kg GO administration partially improved CDDP-induced adverse alterations in all the assayed biochemical parameters ($P \leq 0.05$) compared with those in rats in the CDDP group. However, levels of all the assayed serum biochemical

parameters in rats in the CDDP-GO₅₀ group were higher than those in rats in the control group (Table 1).

3.3. Hepatic lipid peroxidation and antioxidant status

Cisplatin significantly increased the levels of oxidation indicators, including MDA and NO levels ($P \leq 0.05$). Furthermore, CDDP significantly reduced hepatic antioxidant potency, as evidenced by the reduction in GSH level and T.SOD and CAT activities compared with those in control rats (Table 2).

Administration of GO significantly restored the hepatic antioxidant capacity in rats in the CDDP-GO₁₀₀ group ($P \leq 0.05$) to the level present in rats in the control group (Table 2). Moreover, rats in the CDDP-GO₅₀ group showed significantly improved antioxidant status ($P \leq 0.05$); however, this improvement was lower than that in rats in the CDDP-GO₁₀₀ group.

3.4. Renal lipid peroxidation and antioxidant status

Rats in the CDDP group showed significantly increased levels of renal oxidative stress markers, including MDA and NO ($P \leq 0.05$), and significantly decreased GSH level and T.SOD and CAT activities ($P \leq 0.05$) compared with rats in the control group (Table 2). In rats in the CDDP-GO₁₀₀ group, GO administration significantly restored the assayed antioxidant parameters to the normal levels. In rats in the CDDP-GO₅₀ group, GO administration significantly decreased MDA and NO levels ($P \leq 0.05$) and significantly increased GSH level and T.SOD and CAT activities ($P \leq 0.05$) compared with those in rats in the CDDP group.

Table 1
Serum enzyme activities and biochemical marker levels in rats in the control and treated groups.

Parameter	Experimental groups			
	Control	CDDP	CDDP-GO ₅₀	CDDP-GO ₁₀₀
AST (U/L)	50.12 ± 2.34 ^c	116.30 ± 6.54 ^a	70.24 ± 2.34 ^b	53.32 ± 2.34 ^c
ALT (U/L)	29.89 ± 1.49 ^c	75.84 ± 2.92 ^a	44.44 ± 1.49 ^b	31.82 ± 1.49 ^c
ALP (U/L)	62.14 ± 1.48 ^d	115.38 ± 2.64 ^a	77.69 ± 1.48 ^b	69.34 ± 1.48 ^c
LDH (U/L)	320.32 ± 8.09 ^d	515.79 ± 10.59 ^a	413.36 ± 10.59 ^b	354.80 ± 15.61 ^c
Uric acid (mg/dL)	23.75 ± 1.06 ^c	69.99 ± 4.08 ^a	36.62 ± 1.91 ^b	28.34 ± 2.01 ^c
Urea (mg/dl)	19.40 ± 1.49 ^c	68.66 ± 3.66 ^a	39.74 ± 1.37 ^b	24.31 ± 2.85 ^c
Creatinine (mg/dl)	1.18 ± 0.08 ^c	3.93 ± 0.15 ^a	1.69 ± 0.12 ^b	1.28 ± 0.09 ^c
IL-6 (pg/ml)	80.40 ± 3.30 ^c	756.50 ± 34.85 ^a	469.50 ± 34.85 ^b	142.38 ± 8.71 ^c

Means indicated by different superscripted letters within the same row differ significantly at $P \leq 0.05$.

Data are expressed as mean ± SE ($n = 8$).

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDDP, cisplatin; GO, garlic oil; IL-6, interleukin-6; LDH, lactate dehydrogenase.

Table 2
Hepatic and renal lipid peroxidation, oxidative stress markers and antioxidant parameters in rats in the control and treated groups.

Parameter	Experimental groups			
	Control	CDDP	CDDP-GO ₅₀	CDDP-GO ₁₀₀
Hepatic lipid peroxidation and oxidative stress markers				
MDA (nmol/g tissue)	26.37 ± 1.86 ^c	75.58 ± 3.07 ^a	57.64 ± 4.48 ^b	33.60 ± 1.86 ^c
NO (μmol/g tissue)	49.33 ± 2.99 ^c	96.83 ± 2.95 ^a	75.37 ± 2.51 ^b	53.36 ± 2.99 ^c
GSH (μmol/g tissue)	0.32 ± 0.02 ^a	0.19 ± 0.01 ^b	0.23 ± 0.02 ^b	0.31 ± 0.01 ^a
T.SOD (U/g tissue)	127.75 ± 3.99 ^a	46.52 ± 1.28 ^d	89.12 ± 3.59 ^c	109.99 ± 3.32 ^b
CAT (U/g tissue)	45.26 ± 3.16 ^a	24.78 ± 2.24 ^c	34.94 ± 2.52 ^b	42.37 ± 2.52 ^{ab}
Renal lipid peroxidation and oxidative stress markers				
MDA (nmol/g tissue)	72.20 ± 2.97 ^c	141.88 ± 5.02 ^a	95.25 ± 4.39 ^b	77.93 ± 3.09 ^c
NO (μmol/g tissue)	50.79 ± 1.41 ^c	93.01 ± 3.80 ^a	70.94 ± 2.51 ^b	55.13 ± 2.59 ^c
GSH (μmol/g tissue)	5.81 ± 0.12 ^a	1.91 ± 0.29 ^d	3.27 ± 0.16 ^c	5.09 ± 0.16 ^b
T.SOD (U/g tissue)	70.39 ± 1.24 ^a	37.46 ± 1.28 ^c	53.59 ± 2.26 ^b	67.49 ± 2.26 ^a
CAT (U/g tissue)	5.16 ± 0.24 ^a	2.73 ± 0.18 ^c	4.28 ± 0.29 ^b	4.87 ± 0.28 ^{ab}

Means indicated using different superscripted letters within the same row differ significantly at $P \leq 0.05$.

Data are expressed as mean ± SE ($n = 8$).

CAT, catalase; CDDP, cisplatin; GO, garlic oil; GSH, reduced glutathione; MDA, malondialdehyde; NO, nitric oxide; T.SOD, total superoxide dismutase.

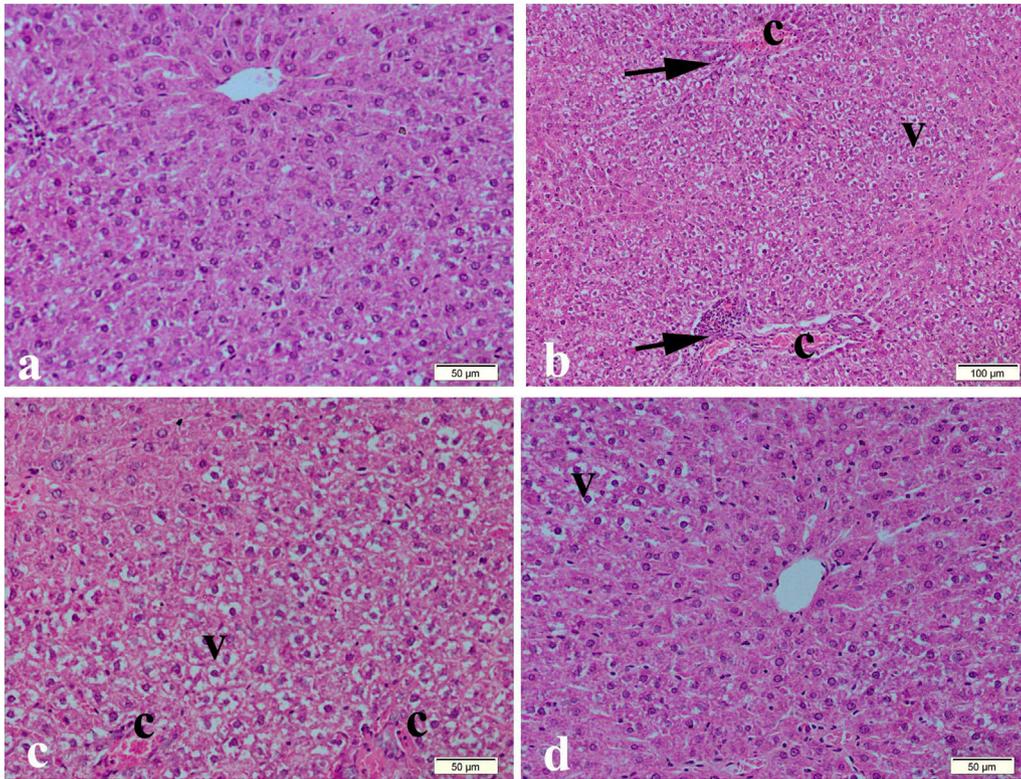


Fig. 1. Histopathological changes in liver tissues stained with H&E; c, congestion; v, vacuolar degeneration. Arrows indicate necrosis and lymphocytic infiltration. Hepatic tissues of rats in the (a) Control, (b) CDDP, (c) CDDP-GO₅₀, and (d) CDDP-GO₁₀₀ groups.

3.5. Liver histopathology

Figure 1a shows the normal histological architecture of hepatic lobules, with polyhedral hepatic cells arranged radially in cords around

the central veins, in rats in the control group. Liver sections of rats in the CDDP group showed blood vessel congestion, severely diffused vacuolar degeneration, hepatocyte necrosis, and inflammatory cell infiltration (Fig. 1b). In contrast, liver revealed marked decreases in

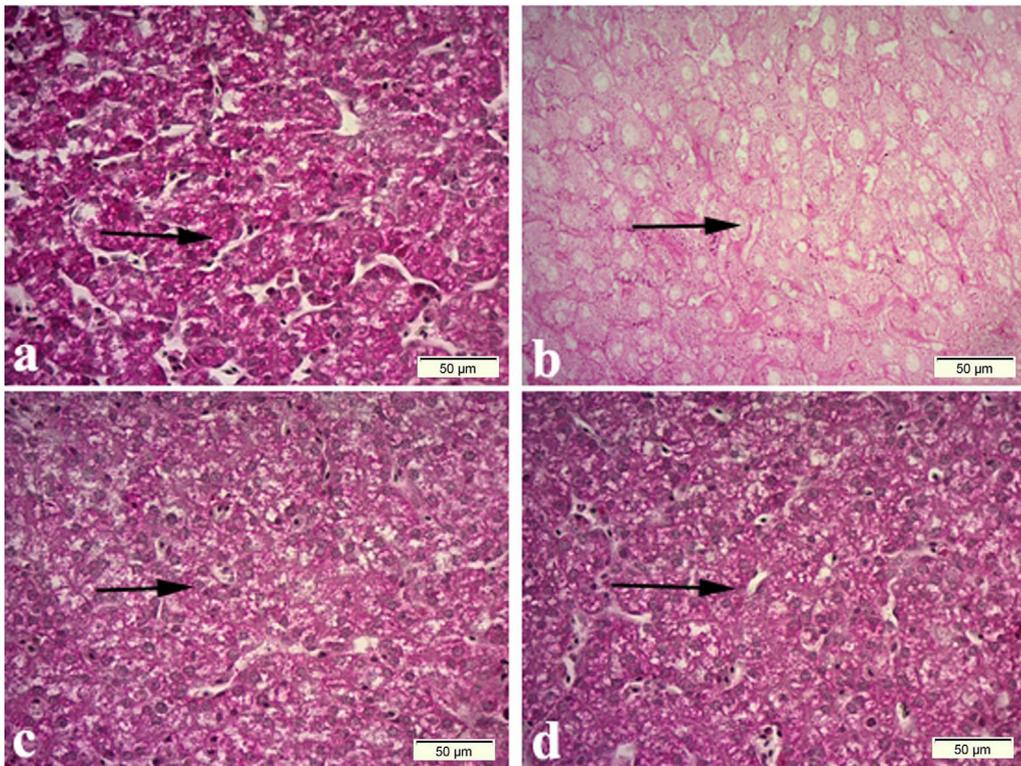


Fig. 2. Histochemical analysis of hepatic tissues stained with PAS; Arrows indicate PAS positivity. Liver tissues of rats in the (a) Control group (showing intense staining), (b) CDDP group (showing marked a reduction in PAS-positive areas), (c) CDDP-GO₅₀ (showing partially intense PAS staining), and (d) CDDP-GO₁₀₀ (showing very intense PAS staining).

Table 3

Liver and Kidney histochemical lesion scores (based on PAS stain) of rats in different control and treated groups.

	Experimental groups			
	Control	CDDP	CDDP-GO ₅₀	CDDP-GO ₁₀₀
Hepatic lesion score				
Field IntDen	5.57 ± 0.002 ^a	4.27 ± 0.006 ^d	5.36 ± 0.007 ^c	5.53 ± 0.003 ^b
Renal lesion score				
Tubular necrosis	0.00 ± 0.00 ^d	5.00 ± 0.00 ^a	3.00 ± 0.26 ^b	2.10 ± 0.23 ^c
Hyaline cast formation	0.00 ± 0.00 ^d	5.00 ± 0.00 ^a	3.10 ± 0.23 ^b	1.90 ± 0.23 ^c
Brush border loss	0.00 ± 0.00 ^d	5.00 ± 0.00 ^a	3.10 ± 0.23 ^b	2.10 ± 0.23 ^c

Means indicated with different superscripted letters within the same row differ significantly at $P \leq 0.05$.

Data are expressed as log mean value ± SE.

CDDP, cisplatin; GO, garlic oil; IntDen, integrated density.

degenerative changes of the hepatocytes and mild congestion of blood vessels. Mild to moderate vacuolar degeneration of hepatocytes with minimal focal necrosis of some other cells were observed in CDDP-GO₅₀ (Fig. 1c). Liver sections of rats in the CDDP-GO₁₀₀ group showed no pathological changes, except mild hepatocyte vacuolar degeneration (Fig. 1d).

In the control group, liver sections of rats stained with PAS showed normal morphology and intense eosinophilic staining of hepatocytes (Fig. 2a). PAS staining of liver sections of rats in the CDDP group showed moderate-to-severe reduction in the number of PAS-stained hepatocytes (Fig. 2b). Liver sections of rats in the CDDP-GO₅₀ group showed intense PAS staining in large areas of the liver parenchyma (Fig. 2c). Liver sections of rats in the CDDP-GO₁₀₀ group showed intense PAS staining of most hepatic cells (Fig. 2d). These results were proven by performing statistical analysis of liver histochemical reaction, as shown in Table 3. Rats in the CDDP group showed a significant decrease

in field IntDen value compared with rats in the control group. In contrast, rats in the CDDP-GO₅₀ and CDDP-GO₁₀₀ groups showed significantly higher field IntDen values than rats in the CDDP group.

3.6. Kidney histopathology

Histological examination of the kidney sections of rats in the control group showed normal glomeruli with normal intact capillary tufts. Rats in the control group showed a normal histological structure for both the cortex and medulla (Fig. 3a). Kidney sections of rats in the CDDP group showed extensive multifocal-to-diffuse cortical tubular degeneration and necrosis. Renal tubules, especially proximal convoluted tubules, of these rats showed swelling, brush border loss, tubular cell necrosis, and tubular nuclear pyknosis. Interstitial tissue of these rats showed severe intertubular blood vessel congestion, with leukocyte infiltration. Moreover, kidney sections of rats in the CDDP group showed hyaline casts in the lumen of majority of cortical renal tubules (Fig. 3b). Kidney sections of rats in the CDDP-GO₅₀ group showed partial improvement in the histological structure of renal tubules compared with that of rats in the CDDP group. Moreover, renal tubules of these rats showed slight swelling, with the presence of hyaline casts. Renal interstitial tissue of these rats did not show obvious fibrous hyperplasia but showed limited inflammatory cell infiltration and some brush border loss (Fig. 3c). Kidney sections of rats in the CDDP-GO₁₀₀ group showed normal renal tissue structure, with swelling in the renal tubules, and normal renal interstitial tissue. Moreover, kidney sections of these rats showed intact brush border in the proximal tubules, with mild vacuolation in the cytoplasm of renal cells (Fig. 3d).

In the control group, kidney sections stained with PAS showed normal morphology and intense eosinophilic staining of the glomerular and tubular basement membranes (Fig. 4a). PAS staining of kidney sections of rats in the CDDP group showed moderate necrosis in the renal tubules, loss of brush border, and no staining of the glomerular and

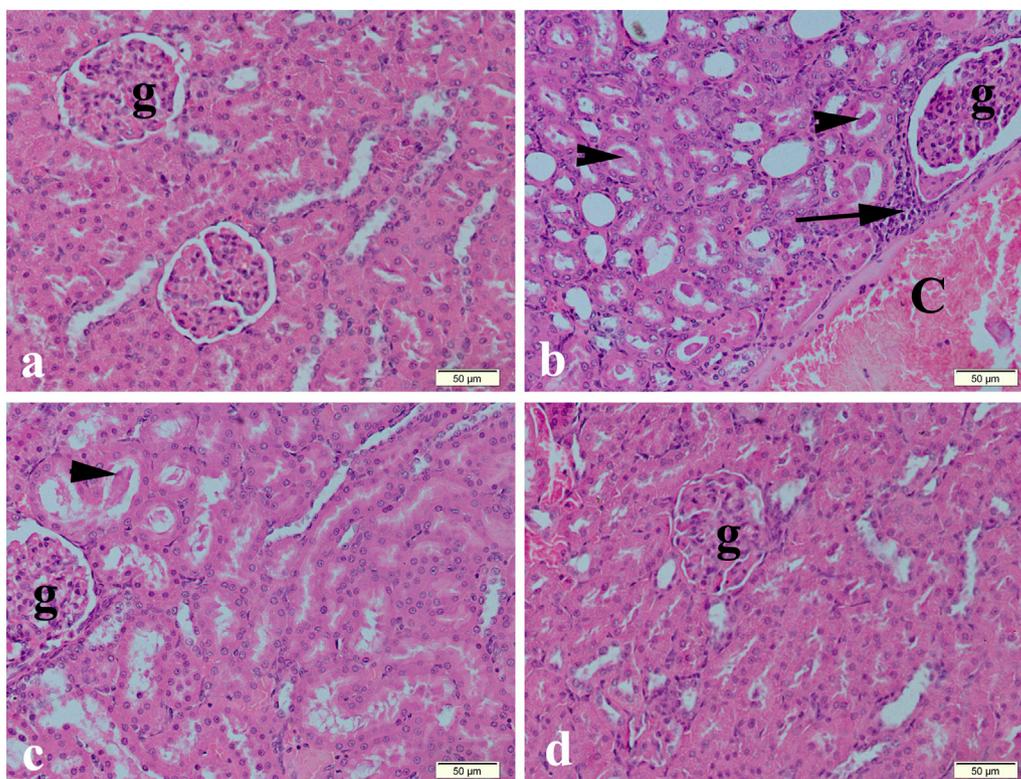


Fig. 3. Histopathological changes of kidney tissues stained with H&E; Arrows indicate leukocyte infiltration, and arrowheads indicate hyaline casts; C, congestion; g, glomeruli. Renal tissues of rats in the (a) Control group (showing normal renal tubules), (b) CDDP group, (c) CDDP-GO₅₀ group, d. CDDP-GO₁₀₀ group.

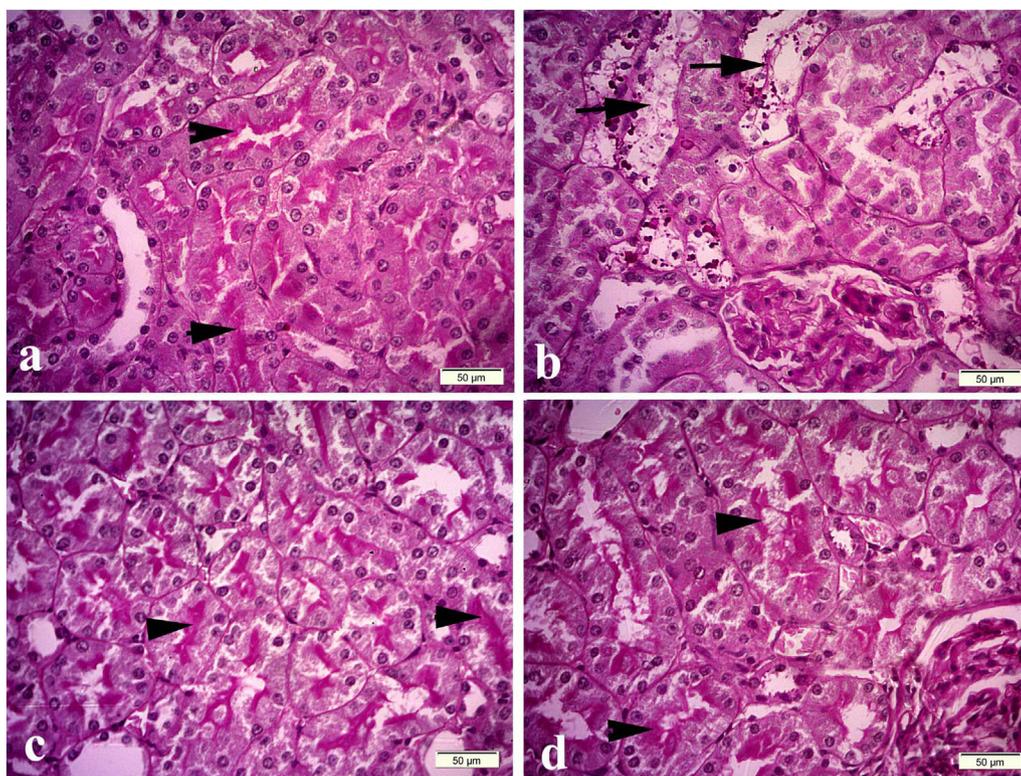


Fig. 4. Histochemical analysis of kidney tissues stained with PAS. Arrows indicate necrosis of renal tubules, and arrowheads indicate brush border of proximal convoluted tubules. Renal tissues of rats in the (a) Control, (b) CDDP, (c) CDDP-GO₅₀, and (d) CDDP-GO₁₀₀ groups.

tubular basement membranes (Fig. 4b). PAS staining of kidney sections of rats in the CDDP-GO₅₀ group showed partial improvement, with intact brush border and intense staining of the tubular basement membrane (Fig. 4c). Renal tubules of rats in the CDDP-GO₁₀₀ showed intense PAS staining of both the tubular and glomerular basement membranes, with intact brush border (Fig. 4d). These results were proven by performing statistical analysis of renal tissue lesion scores, as shown in Table 3. Rats in the CDDP group had a significantly high renal lesion score compared with rats in the control group. In contrast, rats in the CDDP-GO₅₀ and CDDP-GO₁₀₀ groups showed a significant decrease in the renal lesion score compared with rats in the CDDP group.

4. Discussion

Cisplatin exerts deleterious effects on renal and hepatic tissues. In the present study, we evaluated the toxic effect of CDDP on the liver and kidneys and assessed the efficacy of two different doses of GO in alleviating CDDP-induced toxicity. DADS is a degradation product of alliin present in garlic and exerts many pharmacological effects such as antioxidant, anti-inflammatory, and antiapoptotic effects (Abdel-Daim et al., 2017). Ko et al. (2017) found that DADS alleviated acetaminophen-induced nephrotoxicity by attenuating the upregulation of nuclear factor- κ B (NF- κ B), cyclooxygenase-2, and tumor necrosis factor- α (TNF- α) expression in the kidneys and by inhibiting microsomal cytochrome P450 2E1 expression in both the liver and kidneys. Moreover, DADS alleviates cisplatin-induced nephrotoxicity in rats (Chiarandini Fiore et al., 2008). Furthermore, DADS treatment significantly enhances GSH and glutathione-S-transferase (GST, EC 2.5.1.18) activities in the rat liver and kidneys and alleviates CDDP-induced hepatorenal toxicity (Dwivedi et al., 1996).

In the present study, CDDP injection significantly increased the activities of hepatic ALT, AST, ALP, and LDH, which was consistent with the results of previous studies (Abdellatif et al., 2017; Chirino and Pedraza-Chaverri, 2009). CDDP may induce hepatotoxicity by inducing

the development of oxidative stress by promoting the generation of ROS (Chirino and Pedraza-Chaverri, 2009) and by decreasing the activities of antioxidant enzymes (Sadzuka et al., 1992) and levels of non-enzymatic molecules such as GSH (Zhang and Lindup, 1993). In the present study, CDDP-induced oxidative stress was evidenced by a marked increase in hepatic MDA and NO levels and a significant decrease in GSH level and T.SOD and CAT activities in CDDP-treated rats compared with that in control rats. Our results came in agreement with Palipoch et al. (2014). This markedly increases ALT, AST, and ALP levels and induces histopathological alterations in the liver architecture (Arhoghro et al., 2014; Ko et al., 2014; Yuce et al., 2007). CDDP induces inflammatory cell infiltration, hyperplasia, periportal fibrosis, and dilated blood sinusoids. Moreover, CDDP-treated hepatocytes show karyomegaly and pyknotic nuclei, indicating apoptosis in the rat liver (El-Sayyad et al., 2009). Electron microscopic analysis of liver sections of CDDP-treated rats showed degeneration of cellular organelles, including the mitochondria, and vesicular dilation of the rough endoplasmic reticulum (Nasr, 2013). Moreover, CDDP-induced degeneration, necrosis, and apoptosis were accompanied with marked inflammation, as evidenced by the increase in serum levels of cytokines, including IL-6 (Niu et al., 2016). CDDP-induced renal cell injury resulted in the production of damage-associated molecular pattern molecules (DAMPs) which in turn stimulate the release of nuclear factor kappa giving rise to a variety of inflammatory cytokines and then inflammatory reaction took place (Miller et al., 2010).

Cisplatin affects renal cells through the same mechanisms, as evidenced by elevated serum uric acid, urea, creatinine, and IL-6 levels in CDDP-treated rats. Moreover, the present study showed that CDDP induced marked cellular degeneration and necrosis. The same results were recorded by Mahgoub et al. (2017) who confirmed the renotoxic effect of CDDP as evidenced by elevated blood urea nitrogen, creatinine and oxidative/nitrosative stress together with diminished endogenous antioxidants in kidney and finally, raised pro-inflammatory cytokines' levels. Mice i.p. injected with 8 mg/kg CDDP showed significantly

increased serum urea and creatinine levels and renal MDA level and drastically decreased renal SOD activity (Lou et al., 2015). Similarly, CDDP-treated rats showed decreased SOD, CAT, glutathione reductase (GR, EC 1.8.1.7), GST, and GPx activities and significantly increased NF- κ B expression in kidney tissues (Abdel Moneim et al., 2014). Moreover, CDDP induces renal damage by inducing DNA fragmentation and by upregulating TNF- α and IL-6 expression (Yousef and Hussien, 2015). Light microscopic analysis of kidney sections of CDDP-treated rats showed severe tubular degeneration and necrosis, inflammatory cell infiltration, and homogenous eosinophilic cast accumulation in the tubular lumen (Sahu et al., 2014).

The chemopreventive effect of natural antioxidants present in dietary plants, i.e., protection of oxidative damage caused by free radical species, has been gaining attention in recent times (Stavic, 1994). In the present study, GO administration restored CDDP-induced alterations in serum and hepatorenal biochemical parameters in a dose-dependent manner. GO administered at a dose of 100 mg/kg significantly restored CDDP-induced alterations in biochemical markers and antioxidant status in the liver and kidneys. The beneficial role of GO against CDDP-induced oxidative damage observed in the present study may be mediated by the inhibition of MDA and NO, scavenging of free radicals, and increase in GSH and antioxidant enzyme expression. GO scavenges free radicals and has the potential to be a powerful antioxidant. The antioxidant potential of GO was determined by performing 2,2-diphenyl-1-picrylhydrazyl, a NO scavenging agent, and β -carotene bleaching assays (Lawrence and Lawrence, 2011). Aged garlic extract (20% aqueous ethanol) exerts an ameliorative effect against CDDP-induced oxidative stress and renal damage by exerting antioxidant, anti-inflammatory, and antiapoptotic effects. However, mild inflammatory cell infiltration and proximal tubular cell necrosis have been detected in the renal parenchyma of garlic extract-pretreated rats (Nasr and Saleh, 2014). Treatment with an ethanolic extract of garlic might reduce CDDP-induced nephrotoxicity in rats by decreasing serum levels of kidney biomarkers such as urea, uric acid, creatinine, and urea and by increasing the activities of antioxidant enzymes (Anusuya et al., 2013). In the present study, CDDP treatment induced renal tubular deformities and hepatic degeneration in rats. Similarly, Huang et al. (2019) found severe pathological deterioration of the renal tissue stained with PAS and collected from mice i.p. injected once with 22 mg/Kg B.wt. cisplatin. While, GO treatment improved CDDP-induced hepatorenal injuries. Results of histopathological analysis performed in previous studies also confirm that GO improves CDDP-induced nephrotoxicity and hepatotoxicity (Essam et al., 2014; Razo-Rodriguez et al., 2008; Youssef and Al Shahat, 2015). The antioxidant potential of GO prevents or alleviates ROS-induced damage in hepatic and renal cells, thus decreasing cellular inflammation and maintaining normal serum levels of biomarkers of liver and kidney function.

5. Conclusion

Cisplatin-induced renal and hepatic toxicities may be associated with oxidative damage and lipid peroxidation. A daily GO treatment at 100 mg/kg exerted higher beneficial effects against CDDP-induced hepatorenal oxidative injuries compared with treatment with GO at 50 mg/kg. The antioxidant effect of GO was evidenced by the significant decrease in MDA and NO levels and increase in GSH, T.SOD, and CAT activities in the liver and kidneys.

Garlic oil is available in many counties, and this study proposes the potential use of the whole garlic oil as a food supplement in cancer patients under cisplatin treatment. Garlic oil active constituents could act synergistically to produce the given effect. Further studies are required to compare garlic oil as a whole with its active constituents taken separately.

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Declaration of competing interest

All authors have no competing interests to declare.

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