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Original Article

Biochemical and Histological Study of Rat Liver and Kidney Injury Induced by Cisplatin

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Abstract: Cisplatin is a chemotherapeutic agent widely used in treatment of several cancers. It is documented as a major cause of clinical nephrotoxicity and hepatotoxicity. The purpose of this study was to investigate the involvement of oxidative stress in the pathogenesis of cisplatin-induced liver and kidney injury. Wistar rats were divided into four groups. Group 1 (control) was intraperitoneally (IP) injected with a single dose of 0.85% normal saline. Groups 2, 3 and 4 were IP injected with single doses of cisplatin at 10, 25 and 50 mg/kg body weight (BW), respectively. At 24, 48, 72, 96 and 120 h after injection, BW, levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, malondialdehyde (MDA), and activity of superoxide dismutase (SOD) and histology of the liver and kidney were evaluated. Cisplatin caused a reduction in BW of rats in groups 2, 3 and 4 at all post injection intervals. The levels of serum ALT, AST, BUN and creatinine and MDA of the kidney and liver were markedly increased especially at 48 and 72 h, whereas the activity of SOD was decreased after cisplatin injection. Liver sections revealed moderate to severe congestion with dilation of the hepatic artery, portal vein and bile duct and disorganization of hepatic cords at 50 mg/kg of cisplatin. Kidney sections illustrated mild to moderate tubular necrosis at 25 and 50 mg/kg of cisplatin. Therefore, oxidative stress was implicated in the pathogenesis of liver and kidney injury causing biochemical and histological alterations. (DOI: 10.1293/tox.26.293; J Toxicol Pathol 2013; 26: 293–299)

Key words: nephrotoxicity, hepatotoxicity, cisplatin, oxidative stress

Introduction

Cis-diamminedichloroplatinum, or cisplatin, is an important chemotherapeutic agent used for the treatment of various cancers, such as ovarian and testicular cancer[1]. Cisplatin is a divalent, inorganic, water-soluble, platinum-containing complex. After intravenous injection, cisplatin has an initial plasma elimination half-life of 25–50 min in humans. The concentrations of total bound and unbound cisplatin, which have a half-life of ≥24 h in humans, drop thereafter. More than 90% of platinum in the blood is covalently bound to plasma proteins, and after administration of the drug, high levels of cisplatin can be found in tissues of the kidneys, liver, intestines and testes. During the first 6 h after administration, only a small amount of cisplatin is excreted by the kidneys. Up to 25% and 43% of the administered dose is recovered in the urine at 24 h and 5 days, respectively. Biliary and intestinal excretion is minimal[2].

Despite its use as a chemotherapeutic agent, cisplatin exerts serious side effects involving tissues in several organs, including the kidneys and liver[3,4]. Only days after initiating treatment, approximately one-third of cisplatin-treated patients exhibited reduced glomerular filtration rates[5]. The underlying mechanism of nephrotoxicity and hepatotoxicity induced by cisplatin remains incompletely understood. Oxidative stress has been implicated in the pathogenesis of kidney injury induced by cisplatin through increasing reactive oxygen species (ROS), whereas the pathogenicity of the liver injury has not been fully elucidated. Cisplatin-induced liver damage at high doses is characterized by a damaged liver parenchyma. Histological analysis of liver tissue has revealed cytoplasmic changes, especially around cells of the central vein, hepatocellular vacuolization and sinusoidal dilatations[6]. Cisplatin causes a marked reduction in renal function characterized by significant increases in serum blood urea nitrogen (BUN) and creatinine levels. The drug can also cause impairment of liver function characterized by significant increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[7]. Cisplatin at different concentrations has shown varying degrees of functional change and pathological alteration of liver and kidney tissues[8–10]. The aim of the present study was to examine the involvement of oxidative stress in the pathogenesis of cisplatin-induced liver and kidney injury.

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Materials and Methods

Chemicals

Cis-diamminedichloroplatinum (II), Bradford assay kits, and 10% neutral buffered formalin solution were purchased from Sigma-Aldrich Chemical Company (USA). Thiobarbituric acid reactive substances (TBARS) and SOD assay kits were obtained from Cell Biolabs, Inc. (San Diego, CA, USA). All other chemicals were of analytical grade.

Animals

Twelve male Wistar rats (*Rattus norvegicus*) aged 5 weeks and weighing 150–170 g were obtained from the Animal House Division, Faculty of Science, Prince of Songkla University, Thailand. All animal procedures were reviewed and approved (Protocol number: 004/2012) by the Animal Ethics Committee, Walailak University, based on the Code of Practice for the Care and Use of Animals for Scientific Purposes, National Committee for Research Animal Development, National Research Council of Thailand. Rats were maintained in stainless-steel cages under constant conditions of temperature (23 ± 2ºC), relative humidity (50–60%), and lighting (12 h light/dark cycles). Animals were provided with a standard commercial rat diet and distilled water. They were acclimatized and closely monitored under laboratory conditions for 1 week before the researchers performed the experiment.

Experiment design

Wistar rats were randomly divided into four groups of 3 animals each. Group 1 (control) was intraperitoneally (IP) injected with a single dose of 0.85% normal saline. Groups 2, 3 and 4 were IP injected with single doses of cisplatin at concentrations of 10, 25 and 50 mg/kg body weight (BW), respectively. Before collection of specimens, rats were weighed at 24, 48, 72, 96 and 120 h post-injection. Then, rats were anesthetized by IP injection of thiopental sodium (50 mg/kg BW), and blood samples were collected in clot activator tubes. The rats were euthanized more that 120 h post injection with an overdose of thiopental sodium (100 mg/kg BW). The abdominal cavities of the rats were opened, and the liver and kidneys were immediately collected and washed in cold normal saline.

Biochemical analysis

Measurement of serum ALT, AST, BUN and creatinine: Blood samples were centrifuged at 3000 rpm for 5 min. Sera were collected, and the levels of ALT, AST, BUN, and creatinine were measured using a Cobas Mira Plus CC Chemistry Analyzer (Switzerland).

Determination of MDA level: Liver and kidneys were resuspended at 50 mg/mL in phosphate buffered saline (PBS) containing 1X butylated hydroxytoluene (BHT), homogenized on ice and centrifuged at 10000 × g for 5 min to collect supernatant. In accordance with the protocol of an OxiSelect^{TM} TBARS Assay Kit (Cell Biolabs, Cat No.: STA-330), 100 µL of samples or MDA standard was added to separate microcentrifuge tubes, and then 100 µL of the SDS lysis solution was added and mix thoroughly. The samples were then incubated for 5 min at room temperature, 250 µL of thiobarbituric acid (TBA) reagent was added, and then each tube was closed and incubated at 95°C for 60 min. The tubes were then removed and cooled to room temperature in an ice bath for 5 min. All sample tubes were then centrifuged at 3000 rpm for 15 min, the supernatant was removed from the samples, and then finally 200 µL of samples and MDA standard was transferred to a 96-well microplate compatible with a spectrophotometric plate reader. The absorbance was read at 532 nm.

Determination of SOD activity: Liver and kidneys were homogenized at 50 mg/ml in cold 1X lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA) and centrifuged at 12000 × g for 10 min, and the supernatant was collected for analysis. In Accordance with the protocol of an OxiSelect^{TM} SOD Activity Assay Kit (Cell Biolabs, Cat No.: STA-340), 20 µL of samples, 5 µL of xanthine solution, 5 µL of chromagen solution, 5 µL of 10X SOD assay buffer and 50 µL of deionized water were added (total volume of 90 µL) to a 96-well microplate, and then 10 µL of pre-diluted 1X xanthine oxidase solution was added to each well. The samples were then mixed well and incubated for 1 h at 37ºC. The absorbance was read with a spectrophotometric plate reader at 490 nm.

Determination of protein: Protein content was estimated by Bradford assay (Sigma, USA) using bovine serum albumin (BSA) as the standard.

Histological examination and grading

Liver and kidneys were preserved in 10% neutral buffered formalin solution for 24 h and washed with 70% ethanol. Tissues were then placed in small metal caskets, stirred by a magnetic stirrer, dehydrated using alcohol series from 70% to 100% alcohol and embedded in paraffin using an embedding machine. Paraffin blocks were sectioned using a rotary ultra microtome, distributed onto glass slides and then dried overnight. Slides were observed under a light microscope after being stained with hematoxylin and eosin (H&E) dyes and mounted. Two pathologists performed the histopathological grading using a semiquantitative scale: normal = 0, mild = <25%, moderate = 25–50% and severe = >50% of affected area.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Differences between groups were determined by one-way analysis of variance (ANOVA). Post hoc testing was performed for intergroup comparisons using the Least Significant Difference (LSD) test, and a P value <0.05 was considered significant.

Results

Effect of cisplatin on body weight

Cisplatin caused a reduction in rat BW (Fig. 1). At 96 and 120 h, rats treated with 10 mg/kg of cisplatin had significantly reduced BWs ($P<0.05$) compared with rats at 24, 48 and 72 h. At doses of 25 and 50 mg/kg, rats at 72 h had significantly decreased BWs ($P<0.05$) compared with rats at 24 and 48 h; at 96 h, rats had significantly decreased BWs compared with rats at 24, 48, and 72 h ($P<0.05$); and at 120 h, rats had significantly decreased BW ($P<0.05$) compared with rats at 24, 48, 72 and 96 h.

Effect of cisplatin on levels of serum ALT, AST, BUN, and creatinine

ALT and AST are commonly used as liver function biomarkers. At 48 h, all cisplatin-treated groups had significantly increased ALT levels ($P<0.05$) compared with groups at 24 h; the levels decreased continuously until 120 h (Fig. 2A). Groups treated with cisplatin at concentrations of 10, 25 and 50 mg/kg had significantly increased ALT levels ($P<0.05$) at 24, 48, 72, 96 and 120 h compared with the control group. All cisplatin-treated groups had continuously in-

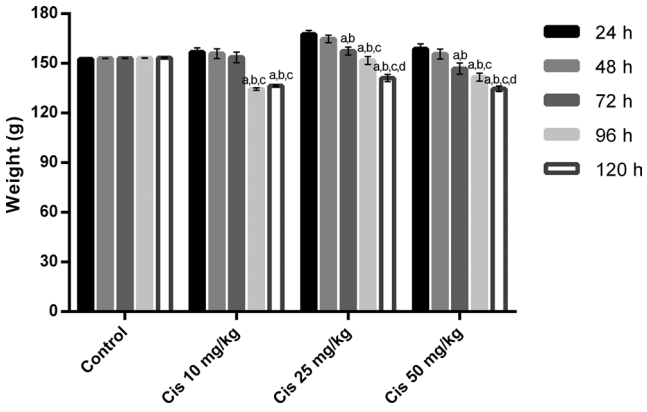

Fig. 1. Effects of cisplatin on BW of Wistar rats. Data were expressed as the mean ± SD (n = 3). [a] $P<0.05$ compared with 24 h. [b] $P<0.05$ compared with 48 h. [c] $P<0.05$ compared with 72 h. [d] $P<0.05$ compared with 96 h.

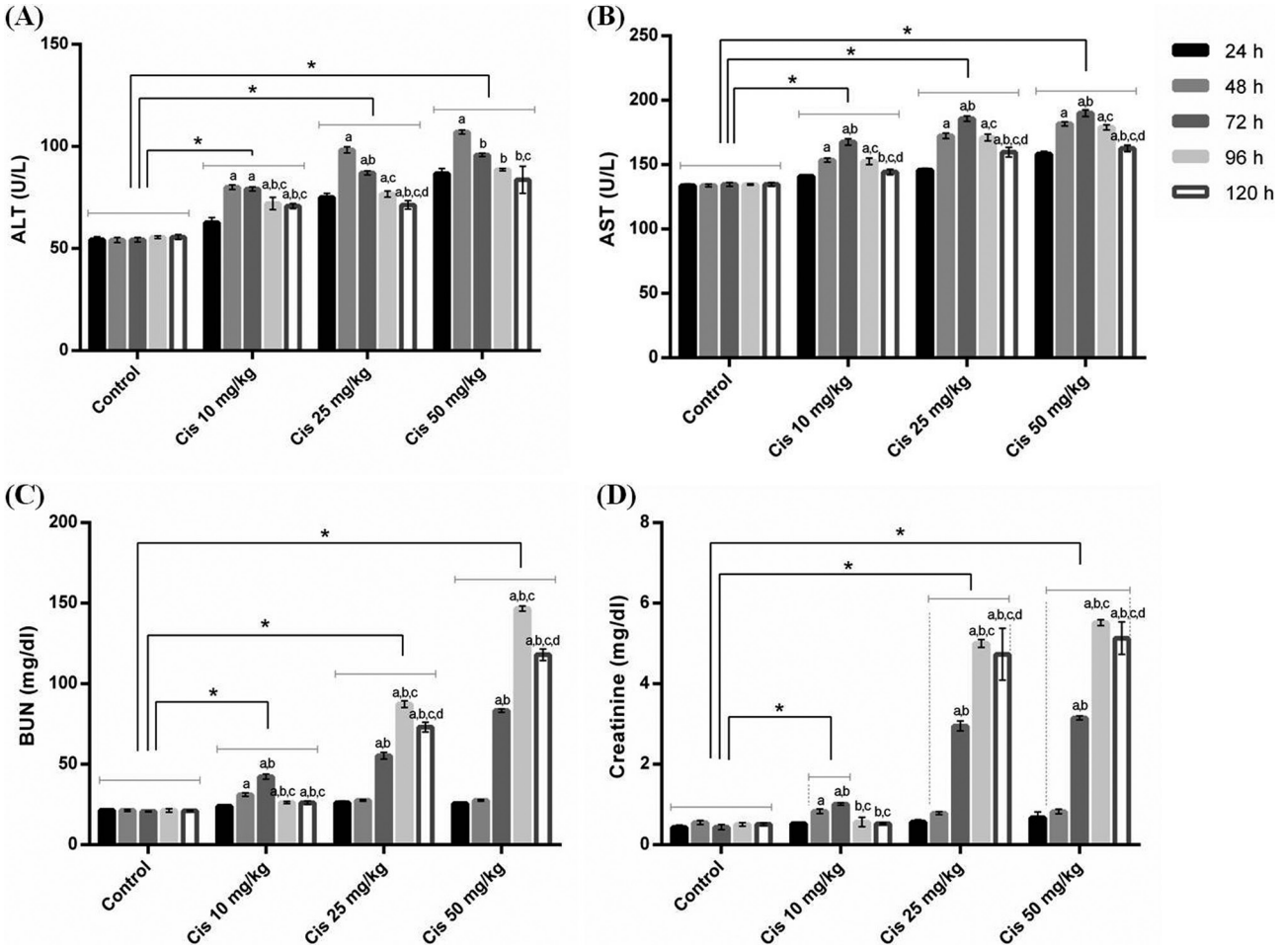

Fig. 2. Effects of cisplatin on ALT, AST, BUN and creatinine levels of Wistar rats. Data were expressed as the mean ± SD (n = 3). [a] $P<0.05$ compared with 24 h. [b] $P<0.05$ compared with 48 h. [c] $P<0.05$ compared with 72 h. [d] $P<0.05$ compared with 96 h. * $P<0.05$ compared with control group.

creased AST levels from 24 h to 72 h and then continuously reduced levels until 120 h (Fig. 2B). Similarly, the groups treated with cisplatin at the concentrations of 10, 25 and 50 mg/kg had significantly increased AST levels ($P<0.05$) at 24, 48, 72, 96 and 120 h compared with the control group. Consequently, cisplatin is capable of causing liver function alterations, as indicated by markedly increased ALT and AST levels, especially 48 and 72 h after treatment.

The 10 mg/kg cisplatin-treated group had continuously increased BUN levels from 24 h to 72 h and reduced BUN levels at 96 and 120 h. The 25 and 50 mg/kg cisplatin-treated groups had continuously increased BUN levels from 24 h to 96 h and decreased levels at 120 h (Fig. 2C). The groups treated with cisplatin at the concentrations of 10, 25 and 50 mg/kg at 24, 48, 72, 96 and 120 h had significantly increased BUN levels ($P<0.05$) compared with the control group. The 10 mg/kg cisplatin-treated group had continuously increased creatinine levels from 24 h to 72 h and reduced levels at 96 and 120 h (Fig. 2D). The 25 and 50 mg/kg cisplatin-treated groups had continuously increased creatinine levels from 24 h to 96 h and decreased levels at 120 h. The 10 mg/kg cisplatin-treated group at 48 and 72 h and the 25 and 50 mg/kg groups (except at 24 h) had markedly increased creatinine levels compared with control group. Therefore, cisplatin is capable of causing kidney function changes, as indicated by increased BUN and creatinine levels, especially 72 and 96 h after treatment.

Effect of cisplatin on MDA level and SOD activity

All concentrations (10, 25 and 50 mg/kg BW) of cisplatin significantly increased MDA levels ($P<0.05$) both in the liver and kidneys compared with the control group, as shown in Fig. 3A. Cisplatin at the concentrations of 25 and 50 mg/kg BW resulted in a marked reduction in SOD activities ($P<0.05$) both in the liver and kidneys compared with the control group (Fig. 3B).

Effect of cisplatin on histology of the liver

The normal microscopic architecture of the liver is composed of hexagonal lobules and acini. Hexagonal lobules are centered on the central vein (CV) and have a portal triad containing branches of the portal vein (PV), hepatic artery (HA) and bile duct (BD). At 120 h after administration of cisplatin at 10 mg/kg, liver sections revealed mild congestion of the CV and mild disorganization of hepatic cords (Fig. 4B). Liver sections from treatment with a higher dose of cisplatin (25 mg/kg) exhibited mild to moderate congestion of the HA, sinusoids and PV, with dilatation of the PV and moderate disorganization of hepatic cords (Fig. 4C). In addition, some hepatocytes were dissociated from hepatic cords, indicating liver injury. Sections treatment with cisplatin at 50 mg/kg showed moderate to severe congestion of the HA, sinusoids and PV, with dilation of the HA, PV and BD and disorganization of hepatic cords (Fig. 4D). Additionally, fusions of the portal triad were observed, indicating severe destruction of nearby hepatic lobules.

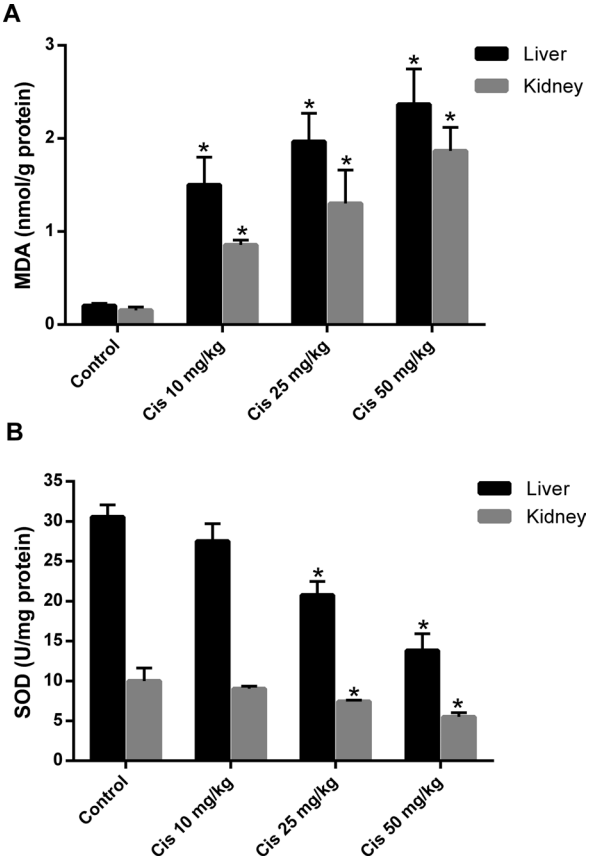

Fig. 3. (A) MDA level and (B) SOD activity in the liver and kidneys at 120 h after administration of cisplatin in Wistar rats. * $P<0.05$ compared with control group.

Effect of cisplatin on histology of the kidneys

Normal histology of rat kidneys (glomeruli, tubules, interstitium and blood vessels) was found in the control group (Fig. 5A). At 120 h after treatment, the 10 mg/kg cisplatin-treated group exhibited proteinaceous casts in the tubular lumen (Fig. 5B). Moreover, detachment of tubular cells from the basement membrane of some tubules was found. Mild to moderate tubular necrosis, especially in the proximal tubule, was observed in kidneys of the 25 and 50 mg/kg cisplatin-treated groups (Fig. 5C and 5D).

Discussion

In the present study, cisplatin was found to cause hepatotoxicity and nephrotoxicity, depending on the concentration of the dose and time after administration. In rat livers, high doses of cisplatin elicited increasing levels of enzymatic biomarkers including ALT and AST, which are associated with degrees of liver damage. We suggest that the elevated serum liver markers were released from the injured liver upon exposure to high doses of cisplatin. In addition, ALT and AST levels were markedly increased, especially at 48 and 72 h after treatment at all concentrations, and this may be caused by excessive accumulation of cisplatin in liver tissues.

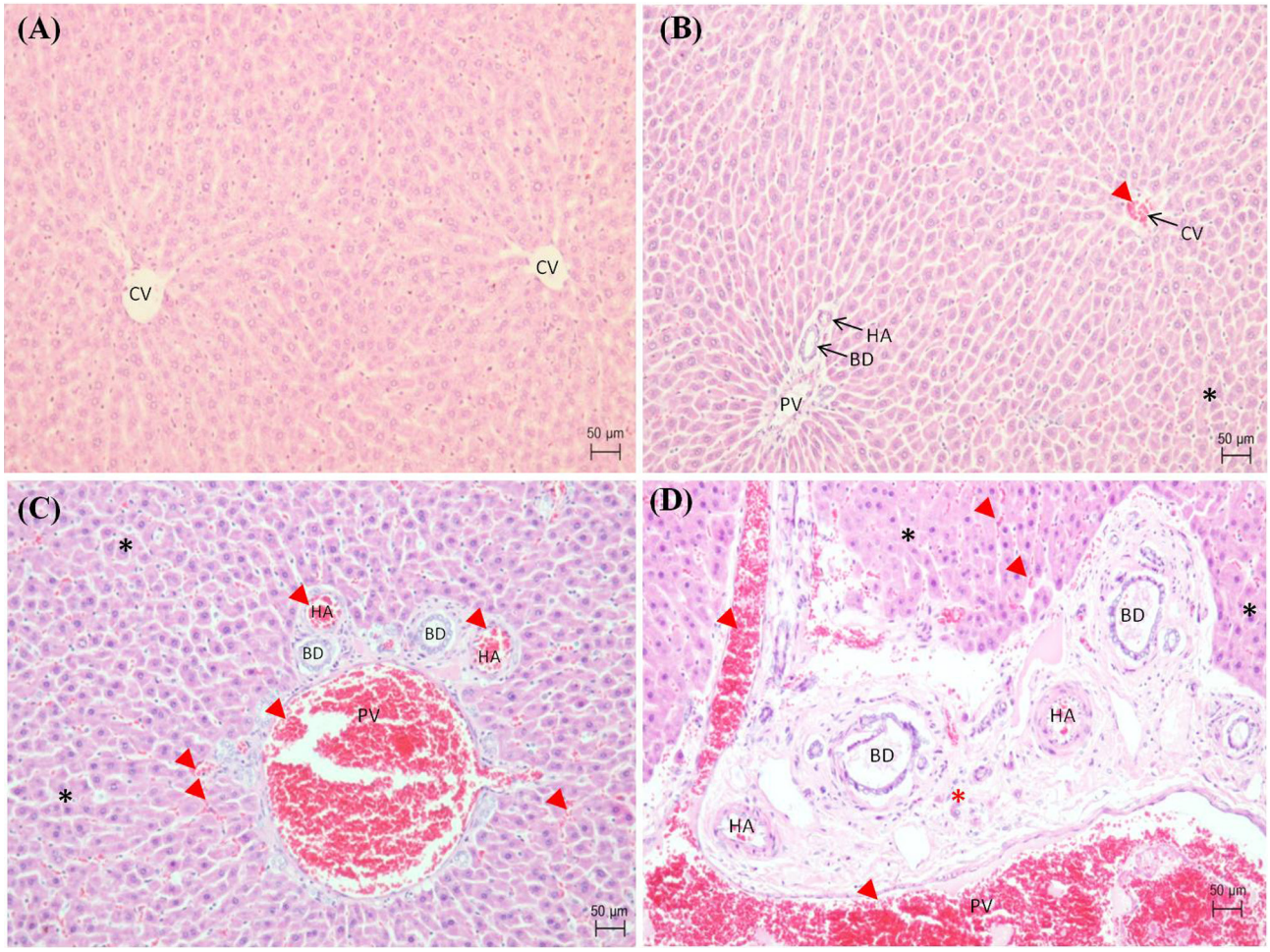

Fig. 4. Histological alterations in the liver at 120 h after administration of cisplatin (H&E staining, 100×). (A) Photomicrograph of a liver in the control group. (B, C, D) Photomicrographs of livers after administration of cisplatin at 10, 25 and 50 mg/kg, respectively. The red arrow indicates congestion. The black asterisk indicates disorganization of hepatic cords. The red asterisk indicates fusion of the portal triad. CV = central vein; HA = hepatic artery; PV = portal vein; BD = bile duct.

Evidence of cisplatin-induced liver injury has been demonstrated by various studies[4,7,11]. Martins *et al.* reported that cisplatin-induced hepatotoxicity involved membrane rigidification, lipid peroxidation, oxidative damage of cardiolipin and protein sulfhydryl groups in Wistar rats[12]. İşeri *et al.* found that a single intravenous injection of cisplatin at a dose of 2.5 mg/kg BW in Sprague-Dawley rats impaired both kidney and liver functions, characterized by significant increases in serum BUN and creatinine levels with a concomitant reduction in calculated creatinine clearance values, and caused significant increases in serum ALT and AST levels compared with a control group[7]. Histological evaluation of kidneys revealed severe degeneration in glomeruli and proximal and distal tubules, and liver revealed acute activation of Kupffer cells, degenerated hepatocytes and moderate enlargement of sinusoids in the cisplatin-treated group[7]. Moreover, the data showed a marked increase in MDA levels, a lipid peroxidation biomarker, and a marked reduction in glutathione (GSH) levels in liver tissues, indicating oxidative stress-induced hepatotoxicity. Hwan Kim *et al.* showed that a single dose of cisplatin IP injected at 45 mg/kg BW in ICR mice can cause liver function impairment, characterized by elevation of AST (328%) and ALT (330%) activities, through the underlying mechanism of cisplatin-induced inflammation[11]. Liao *et al.* demonstrated that cisplatin altered liver function of male albino mice and that this was accompanied by significantly increased ALT activity in mice IP treated once a day for five days with 3.5 mg/kg BW of cisplatin via a mechanism of oxidative stress caused by increased MDA and reduced GSH levels[4].

Our data suggests that cisplatin can cause kidney function impairment via elevation of kidney function biomarker levels including BUN and creatinine, which is associated with kidney pathologies, including proteinaceous casts in the tubular lumen, detachment of tubular cells from the basement membrane, and tubular necrosis, depending on the dose and time of treatment. An *et al.* and Atasayar *et al.* found that the histology of renal sections exhibited remarkable vacuolation, necrosis, desquamation of epithelial cells, and proteinaceous casts in renal tubules after intraperito-

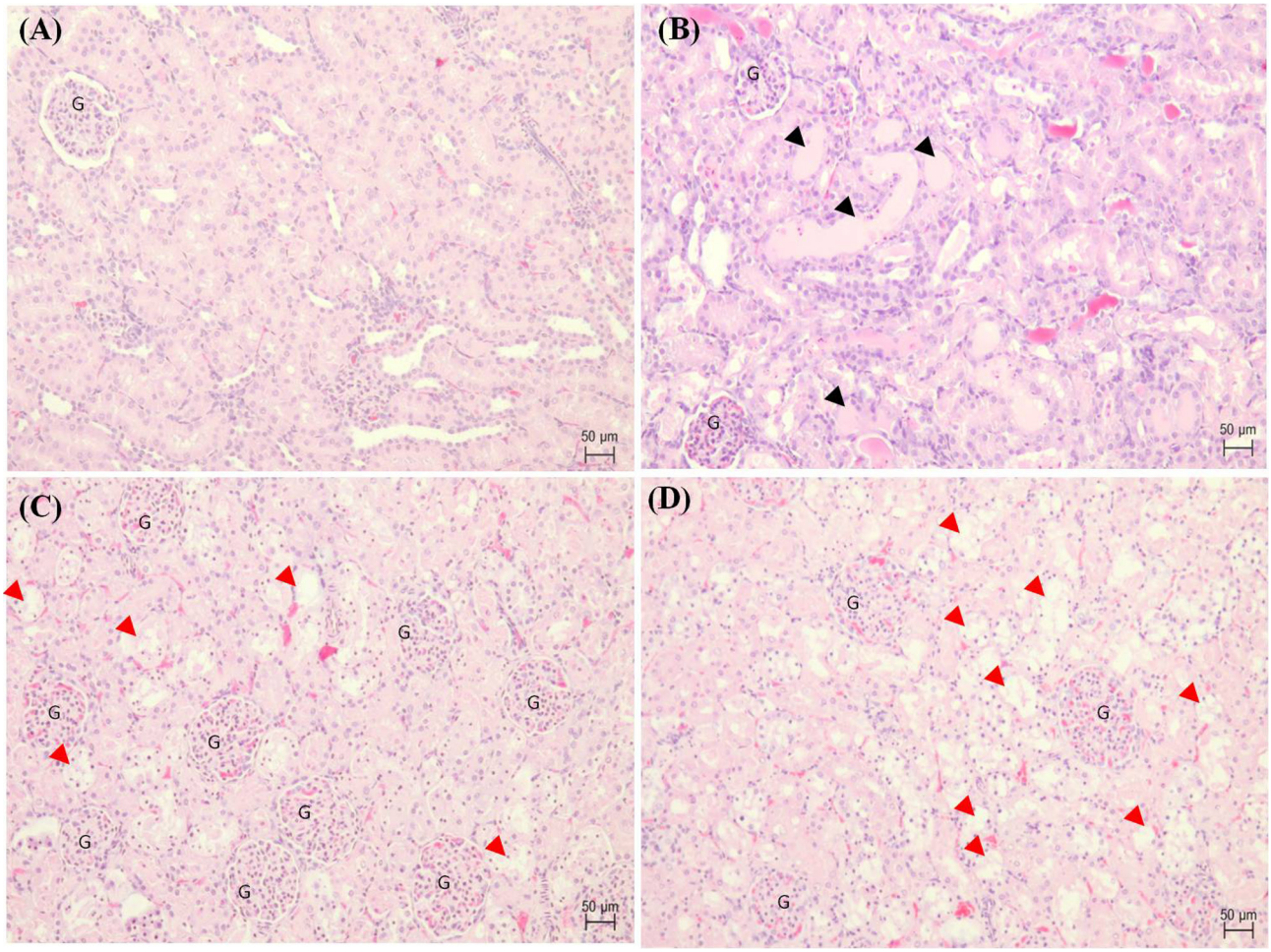

Fig. 5. Histological alterations in the kidneys 120 h after administration of cisplatin (H&E staining, 100×). (A) Photomicrograph of a kidney in the control group. (B, C, D) Photomicrographs of kidneys after administration of cisplatin at 10, 25 and 50 mg/kg, respectively. The black arrow indicates proteinaceous casts in tubular lumen. The red arrow indicates tubular necrosis. G = glomerulus.

neal treatment with single doses of cisplatin at 5 mg/kg BW in ICR mice[10] and 7.5 mg/kg BW in male Sprague-Dawley rats[13]. The toxic effects of cisplatin in the present study were similar to those discovered by Badary *et al*., who found that intravenous cisplatin administration (7 mg/kg BW) caused abnormal kidney function in male Wistar albino rats, as evidenced by markedly increased levels of serum BUN and creatinine compared with a control group[14]. Khan *et al.* and El-Beshbishy *et al.* also found nephrotoxic effects of cisplatin on renal function[9,15]. They suggested the underlying mechanism was cisplatin-induced oxidative stress through elevation of ROS and reactive nitrogen species (RNS), and reduction of the antioxidant defense system[13,16–20]. Cisplatin administered at 12 mg/kg BW in male Swiss albino mice for four days increased the levels of MDA, indicating lipid peroxidation and reduced levels of GSH, catalase, SOD, and glutathione peroxidase (GPx) in the kidneys[9]. Jiang *et al.* demonstrated the possible involvement of ROS, particularly hydroxyl radical, in p53 activation, tubular cell apoptosis and nephrotoxicity in cisplatin treatment for both *in vitro* and *in vivo* models[21]. Antioxidants, or free radical scavengers, have the ability to protect cells and tissues exposed to cisplatin from toxicity. The natural antioxidant naringenin was able to improve cisplatin-induced nephrotoxicity via significant enhancement of enzymatic antioxidant activities including SOD, GPx, and catalase[14]. Green tea consumption revealed marked improvement in reduction of cisplatin-induced oxidative stress via increases in the activities of renal SOD and catalase. We suggested that oxidative stress is one of the most important mechanisms causing hepatic injury as well as renal injury induced by cisplatin via increased lipid peroxidation and reduced activity of endogenous antioxidant.

In conclusion, oxidative stress was implicated in the pathogenesis of kidney injury induced by cisplatin as well as in liver injury**.** Cisplatin causes liver and kidney damage depending on the dose concentration and time after administration. High doses of cisplatin demonstrated kidney function impairment as a result of increased levels of enzymatic biomarkers including ALT and AST, which are associated with the degree of liver damage, and impairment of liver function as a result of elevated biomarker levels, including

BUN and creatinine, which are associated with pathologies of the kidney.

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