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Cisplatin-Induced Oxidative Stress and Hepatorenal Damage: Implications From Preclinical Rat Models For Toxicological Pathology.

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Abstract

CISPLATIN, a cornerstone chemotherapeutic agent, is significantly limited by dose-dependent hepatorenal toxicity. This study comprehensively evaluated cisplatin-induced hepatorenal injury in a rat model using 5, 7, and 10 mg/kg doses, with biochemical, oxidative stress, and histopathological analyses at 3 and 5 days post-treatment. Liver toxicity manifested as dose- and time-dependent elevations in serum ALT and AST at 10 mg/kg by Day 5, correlating with histopathological evidence of hepatocellular necrosis, inflammatory infiltrates, and portal fibrosis. Oxidative stress in the liver was marked by increased lipid peroxidation and a compensatory but insufficient antioxidant response. Conversely, renal toxicity exhibited distinct oxidative dynamics, with elevated MDA and significant SOD depletion, indicating compromised antioxidant defenses. Renal dysfunction was evidenced by dose-dependent increases in serum creatinine and urea alongside histopathological findings of tubular necrosis, interstitial inflammation, and fibrosis. Histopathological scoring confirmed progressive injury, with hepatocellular damage and tubular necrosis peaking at 10 mg/kg by Day 5. Immunohistochemical analysis revealed heightened caspase-3 and TNF-α expression, implicating oxidative stress-mediated apoptosis and inflammation as central mechanisms. These findings highlight organ-specific antioxidant responses, with hepatic SOD activity transiently countering oxidative stress, while renal SOD depletion exacerbated damage. The temporal and dose-dependent progression of injury underscores the need for rigorous clinical monitoring and adjunct therapies, such as antioxidants or targeted drug delivery, to mitigate cisplatin's toxicity without compromising its antitumor efficacy. This study provides critical insights into the mechanistic interplay of oxidative stress and organ damage, informing strategies to enhance therapeutic safety in oncology.

Keywords: Cisplatin, Biochemical changes, Oxidative stress, Hepatorenal damage, Rat.

Introduction

Since its approval in the late 1970s, cisplatin, a platinum-based chemotherapeutic agent, has been a key component in treating many solid malignancies, such as ovarian, testicular, lung, and bladder cancers. Its efficacy comes from its ability to induce DNA cross-linking, causing rapidly dividing cancer cells to undergo apoptosis [1]. However, the dose-dependent toxicity of cisplatin, especially hepatotoxicity and nephrotoxicity, severely limits its therapeutic significance despite its potent anticancer capabilities.

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These side effects undermine the drug's therapeutic potential by compromising the patient's quality of life and requiring dose reduction or discontinuation of treatment [2].

Cisplatin's hepatotoxic effects have been well characterized, and they are principally mediated by the formation of reactive oxygen species (ROS), which disturb cellular homeostasis, cause lipid peroxidation, and activate inflammatory and apoptotic pathways [3]. Histopathological studies have revealed that cisplatin-induced liver injury is characterized by hepatocellular necrosis, inflammatory infiltrates, and architectural disorganization, often exacerbated by the drug's accumulation in hepatic tissues [4]. Similarly, cisplatin-induced nephrotoxicity, which predominantly affects the renal proximal tubules, is a major clinical concern. The drug's accumulation in renal tissues leads to tubular cell apoptosis, inflammation, and oxidative stress, culminating in acute kidney damage [5]. These toxicities are further compounded by activating key apoptotic pathways involving proteins like caspase-3 and p53 [6].

The underlying mechanisms of cisplatin-induced hepatorenal toxicity are compound and multifactorial, with oxidative stress playing a central role. Cisplatin disrupts mitochondrial function, producing excessive ROS and damaging cellular macromolecules, including lipids, proteins, and DNA [7]. This oxidative damage is further exacerbated by the depletion of endogenous antioxidants like glutathione (GSH) and superoxide dismutase (SOD), creating a vicious cycle of oxidative stress and cellular injury [8]. Preclinical studies utilizing rodent models have been instrumental in elucidating these mechanisms, providing valuable insights into potential strategies for mitigating cisplatin-induced organ damage. These include using antioxidants, anti-inflammatory agents, and other cytoprotective compounds that target oxidative stress pathways [9].

Despite significant advances in understanding cisplatin-induced toxicity, the balance between its therapeutic efficacy and adverse effects remains a critical challenge in clinical oncology. While dose adjustments and hydration protocols have been implemented to reduce nephrotoxicity, these measures are often insufficient to prevent hepatotoxicity, highlighting the need for more effective protective strategies [10]. Furthermore, the dose-dependent nature of cisplatin-induced hepatorenal injury underscores the importance of identifying safe therapeutic windows and developing targeted interventions to mitigate organ damage without compromising antitumor efficacy [11].

This work aims to thoroughly assess cisplatin-induced hepatorenal toxicity in a rat model, employing a dose-dependent approach to assess biochemical, oxidative stress, and histopathological changes at two critical time points post-treatment. By analysing serum levels of liver enzymes (ALT and AST) and renal function markers (urea and creatinine), alongside oxidative stress markers (MDA and SOD) and histopathological examinations, this research seeks to elucidate the mechanisms underlying cisplatin-induced organ damage. The results of this work are anticipated to lead to the development of novel strategies for mitigating cisplatin-induced hepatorenal toxicity, thereby enhancing therapeutic outcomes in cancer patients.

Material and Methods

Ethical Approval and Animal Welfare

Every experimental procedure was authorized by the Faculty of Veterinary Medicine's Ethics Committee at Benha University (Approval Number: BUFVTM 05-01-23) and carried out in compliance with the National Institutes of Health's (NIH) guidelines for the care and use of laboratory animals.

Experimental Design

Cisplatin (cis-Diamminedichloroplatinum II), a crystalline compound with a white to yellow-orange appearance, was procured from United Pharmaceuticals, Badr Industrial City, Egypt. The medication was dissolved in sterile saline and given as a single intraperitoneal injection. Blood and tissue samples were collected for biochemical, oxidative stress, and histopathological analyses three and five days after treatment. In a rat model, the toxicological effects of cisplatin on liver and kidney function were assessed over time and dose. The rats were kept in a controlled environment with a 12-hour light/dark cycle and a temperature of 22 ± 2°C. Water and standard laboratory chow were given freely. The animals were acclimated to laboratory conditions for one week before the experiment began to reduce stress and guarantee physiological adaptation.

Forty male 200–250 gm Sprague-Dawley rats were split into four groups at random (n = 10 per group):

-Control Group: Intraperitoneal injection of sterile saline.

-Cisplatin 5 mg/kg Group: Intraperitoneal injection of 5 mg/kg of cisplatin.

-Cisplatin 7 mg/kg Group: Intraperitoneal injection of 7 mg/kg of cisplatin.

-Cisplatin 10 mg/kg Group: Intraperitoneal injection of 10 mg/kg of cisplatin.

Biochemical Analysis

Serum Preparation and Blood Collection

Under anesthesia, cardiac punctures were used to obtain blood samples from the eye's medial canthus [12]. The blood sample was obtained in sterile tubes without the use of anticoagulants. Samples were

centrifuged for 10 minutes at 3000 rpm to extract the serum. The serum was stored at -80°C for further examination as bioassays.

Liver and Kidney Function Tests

As markers of liver function, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and kidney function indicators as serum urea and creatinine levels were measured. Using commercially available diagnostic kits (Biodiagnostic Company, Dokki, Giza, Egypt) we can measure ALT and AST levels following the method described by [13]. Serum level of creatinine and urea were assessed calorimetrically by assay kits provided by the same manufacturer, according to the instructions provided in the kit protocols. Every measurement was carried out three times to guarantee accuracy, and the outcomes were reported in standard units.

Oxidative Stress Analysis

Following euthanasia, liver and kidney tissues from the euthanized animal were removed, thoroughly cleaned in ice-cold phosphate-buffered saline (PBS, pH 7.4) to get rid of blood and debris, and then homogenized in PBS with a mechanical homogenizer. After centrifuging the resultant homogenates for 15 minutes at 4°C at 10,000 rpm, the supernatant was gathered and kept at -80°C for oxidative stress analysis. The concentration of malondialdehyde (MDA), an indicator of lipid peroxidation, was identified in liver and kidney tissue homogenates using a commercially available colorimetric kit (Biodiagnostic Company, Cairo, Egypt; Catalog No. MD 25 29), following the protocol described by [14]. Superoxide dismutase (SOD) activity, an indicator of antioxidant defense, was measured using a specific diagnostic kit (Biodiagnostic Company, Giza, Egypt; Catalog No. SD 25 21), based on the method of [15]. Both assays followed the manufacturer's instructions, expressing the results in the appropriate units.

Histopathological Examination

To preserve morphological integrity, liver and kidney tissues were fixed in 10% neutral-buffered formalin for 24-48 hours each. Tissues were dehydrated using graded ethanol, cleared with xylene, and embedded in paraffin wax. A rotary microtome was used to cut 5 µm-thick sections. Histological changes were assessed using hematoxylin and eosin (H&E) stained tissue sections. Unaware of the treatment groups, two independent pathologists examined stained slides under a light microscope [16].

The purpose of the histopathological scoring system for liver and kidney tissues was to quantitatively measure the extent of cisplatin-induced organ damage. The liver's inflammatory infiltration, sinusoidal changes, and hepatocellular damage were assessed and graded on a scale of 0 to 3, where 0 denotes no damage, 1 mild change, 2 moderate changes, and 3 severe injuries. Tubular dilatation, tubular necrosis, loss of brush border, and cast formation were evaluated and scored on a scale of 0 to 5 in the kidneys, where 0 denoted no damage, 1 denoted involvement of ≤10%, 2 denoted participation of 11–25%, 3 denoted involvement of 26–45%, 4 denoted participation of 46–75%, and 5 denoted involvement of ≥76% [17, 18].

Immunohistochemical

Sections 5 um thick were mounted on slides coated with polylysine. Samples were rehydrated, then processed in citrate buffer (pH 7.6) for 10 minutes in an oven (1200) before being allowed to cool at room temperature. The sections were then cleaned in phosphate-buffered saline (PBS). After that, the pieces were submerged in 0.3% H2O2 for seven minutes before being washed with PBS. Sections were incubated overnight with the primary rabbit polyclonal Caspase-3 antibody (Ab4051, Abcam Ltd, Cambridge, UK). Following a PBS soak, they were incubated for ten minutes at room temperature with biotinylated goat anti-polyvalent. The slides were rinsed with water and dehydrated after mixing with Mayer's hematoxylin for one minute, followed by a chromogen-positive substrate base of fifteen minutes.

Statistical Analysis

The data was presented as a mean ± standard deviation of the mean (SD) for every parameter under investigation. Tukey-Kramer was used for the post hoc test after the various groups were compared using one-way analysis of variance (ANOVA). Version 22 of the SPSS software (Statistical Package for Social Sciences) was used to perform all statistics. Any difference with a p-value less than 0.05 was deemed statistically significant.

Results

Effect of cisplatin on liver at 3rd and 5th day post-treatment

In comparison to the control group, the evaluation of liver function after cisplatin administration showed a notable, dose-dependent rise in serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at Day 3& Day 5. The control group kept its ALT and AST levels within normal limits on Day 3. However, when compared to the control group, the cisplatin-treated groups on Day 3& Day 5 showed a significant increase in both markers, with the 5 mg/kg group demonstrating substantial increases, the 7 mg/kg group demonstrating high significant gains, and the 10 mg/kg group demonstrating very high significant increases (Table 1).

Corresponding to oxidative stress markers in the liver, on Day 3 & Day 5, MDA levels in cisplatin-treated groups revealed significantly higher levels than the control group, indicating a dose-dependent enhancement of lipid peroxidation. The groups that received 5 mg/kg, 7 mg/kg, and 10 mg/kg showed significant increases, highly significant increases, and very high significant increases, respectively, compared to the control group. Additionally, there was significant decrease in SOD levels, indicating oxidative damage caused by cisplatin (Table 2).

Effect of cisplatin on the kidney at the 3rd and 5th day post-treatment

Renal function assessment after cisplatin administration showed a significant dose-dependent increase in serum urea and creatinine levels on day 3 and day 5 compared to the control group. On day 3, the control group maintained urea and creatinine levels within normal ranges. In contrast, the cisplatin-treated groups on day 3 and day 5 showed significant increases in both markers, with the 5 mg/kg group showing substantial gains, the 7 mg/kg group showing very significant increases and the 10 mg/kg group showing very substantial increases when compared to the control group, respectively (Table 3).

Consistent with markers of oxidative stress in the kidney, MDA levels on days 3 and 5 in the cisplatin-treated groups showed significant increases compared to the control group, suggesting a dose-dependent enhancement of lipid peroxidation. The 5 mg/kg group showed significant gains, the 7 mg/kg group showed substantial increases, and the 10 mg/kg group showed significant increases compared to the control group. In contrast, SOD levels decreased. The 5 mg/kg group showed significant decreases, the 7 mg/kg group showed significant decreases, and the 10 mg/kg group showed very substantial reductions when compared to the control group in SOD, respectively (Table 4).

Histopathological and Immunohistochemical Findings

Histopathological analysis of liver tissues revealed dose- and time-dependent degenerative and inflammatory changes following cisplatin administration. In the control group, the liver architecture appeared normal, with intact hepatocyte organization and well-defined portal areas, showing no evidence of cellular degeneration or necrosis. Conversely, the 5 mg/kg group exhibited focal areas of hepatocellular degeneration characterized by hydropic changes and cytoplasmic vacuolization in some hepatocytes. Mild nuclear pyknosis indicated early necrotic changes, while moderate portal inflammation with lymphocytic infiltration around portal tracts was present. The 7 mg/kg group displayed more pronounced hepatocellular necrosis, particularly in periportal areas, with extensive inflammatory infiltrates comprising neutrophils and lymphocytes. Disruption of liver architecture and the beginning stages of fibrosis in portal regions were also noted. The 10 mg/kg group presented marked hepatocellular necrosis, leading to significant loss of hepatic architecture. Extensive inflammatory infiltrate with predominant neutrophils suggested acute inflammation alongside evidence of cholestasis, bile duct hyperplasia, and significant portal fibrosis (Fig. 1). Regarding kidney histopathology, the control group exhibited typical characteristics, with unbroken glomeruli and well-preserved tubular architecture. The 5 mg/kg group revealed early signs of tubular injury, characterized by focal tubular degeneration, hydropic changes in proximal tubular cells, and loss of brush border. Moderate interstitial edema and inflammatory cell infiltrate, primarily lymphocytes, were noted. The 7 mg/kg group showed more pronounced tubular necrosis, especially in proximal convoluted tubules, with extensive cytoplasmic vacuolation and tubular casts. Interstitial inflammation was marked, with neutrophils predominant and signs of mild interstitial fibrosis emerging. Finally, the 10 mg/kg group exhibited severe tubular necrosis, extensive loss of epithelial integrity, and significant interstitial fibrosis, indicating progression towards chronic kidney injury, with glomerular changes potentially including hypercellularity and initial sclerosis. Overall, these findings underscore cisplatin's severe hepatotoxic and nephrotoxic effects, characterized by significant elevations in liver enzymes, histopathological alterations, and oxidative stress markers in both kidney and liver tissues. The data highlights the need to monitor hepatic and renal function during cisplatin treatment carefully (Fig. 2). Immunohistochemical analysis revealed dose- and time-dependent caspase-3 activation. Control livers and kidneys displayed negligible caspase-3 expression (Fig. 3A, E). The 5 mg/kg group exhibited moderate nuclear staining in scattered hepatocytes and renal tubular cells (Fig. 3B, F), intensifying to widespread positivity in the 7 mg/kg (Fig. 3C, G) and 10 mg/kg groups (Fig. 3D, H). Caspase-3 expression correlated with histopathological severity, peaking at Day 5 in the 10 mg/kg group, consistent with extensive necrosis, fibrosis and inflammation. Tumor necrosis factor (TNF) staining in hepatic and renal tissues mirrored histopathological damage. Minimal expression was noted in controls (Fig. 4A, E). The 5 mg/kg group showed focal hepatocytes and tubular epithelial positivity (Fig. 4B, F), which became diffuse in the degenerated tissues of the 7 mg/kg group (Fig. 4C, G). The 10 mg/kg group demonstrated intense TNF staining in necrotic hepatocytes, tubules and inflamed interstitium (Fig. 4D, H), aligning with maximal necrosis and fibrosis at Day 5.

Histopathological Scoring of Liver and Kidney

At Day 3 post-cisplatin treatment, mild histopathological changes were observed in both liver and kidney tissues. In the liver, the 5 mg/kg group shows modest increases in hepatocellular damage (0.9), sinusoidal changes (0.7), and inflammatory infiltration (0.8). In contrast, the 7 and 10 mg/kg groups display progressively higher scores, with the highest dose nearing the upper limit of the scale. In the kidney, early injury is reflected by scores around 1.0–1.3 in the 5 mg/kg group, increasing to moderate damage in the 7 mg/kg group and reaching near-maximal levels in the 10 mg/kg group (Table 5).

By Day 5, both hepatic and renal damages are significantly more pronounced. In all three parameters, liver tissue from the 5, 7, and 10 mg/kg groups shows clear dose-dependent increases (mean scores of approximately 1.1, 1.7, and 2.0, respectively), indicating severe tissue injury at the highest dose. Kidney injury follows a similar trend, with the 5 mg/kg group displaying mild alterations, the 7 mg/kg group showing moderate damage, and the 10 mg/kg group reaching the maximum or near-maximum scores across all four evaluated parameters. These data confirm a progressive, dose- and time-dependent increase in hepatotoxicity and nephrotoxicity following cisplatin administration (Table 6).

Discussion

The present study demonstrates that cisplatin induces significant dose- and time-dependent hepatotoxicity and nephrotoxicity in a rat model, mediated primarily through oxidative stress mechanisms. Our results concur with earlier research highlighting cisplatin's dual organ toxicity, particularly its propensity to generate reactive oxygen species (ROS) and disrupt cellular homeostasis [19,2]. The results provide critical insights into the biochemical and histopathological alterations associated with cisplatin-induced organ damage, underscoring the need for protective strategies to mitigate these adverse effects.

The observed elevations in serum AST and ALT concentrations in cisplatin-treated rats agree with hepatocellular injury, as these enzymes are released into the bloodstream following liver cell damage [20]. The dose-dependent increase in AST and ALT concentrations, particularly in the 10 mg/kg group, suggests that higher doses of cisplatin exacerbate liver injury. This is further supported by histopathological findings, which revealed progressive hepatocellular necrosis, inflammatory infiltrates, and sinusoidal changes in cisplatin-treated groups. The severity of these changes was proportional to the dose and duration of cisplatin exposure, with the 10 mg/kg group showing the most extensive damage by Day 5.

The marked rise in MDA levels and the corresponding fall in SOD activity demonstrate the part oxidative stress plays in cisplatin-induced hepatotoxicity. The dose-dependent elevation of MDA, a lipid peroxidation marker, suggests that cisplatin encourages oxidative damage to cellular membranes. This aligns with earlier research showing that cisplatin causes lipid peroxidation and membrane stiffening in hepatic tissues [21]. The imbalance between ROS generation and antioxidant defense mechanisms in rats treated with cisplatin is further highlighted by the decrease in SOD activity, a crucial antioxidant enzyme. These results imply that, as previously noted [22], oxidative stress is a key player in the pathophysiology of cisplatin-induced liver damage.

Significant increases in serum urea and creatinine levels, which are signs of compromised renal function, were observed in cisplatin-induced nephrotoxicity. The progressive character of cisplatin-induced kidney damage is reflected in the dose-dependent rise in these markers, especially in the 10 mg/kg group. Histopathological analysis further supported these results, which showed interstitial fibrosis, tubular necrosis, and loss of brush border in rats treated with cisplatin. As time and dose increased, so did the degree of renal damage; by Day 5, the group receiving 10 mg/kg showed the most noticeable histopathological changes.

The marked rise in renal MDA levels and the decrease in SOD activity support the idea that oxidative stress plays a part in cisplatin-induced nephrotoxicity. Reduced SOD activity signifies the deterioration of antioxidant defenses, whereas elevated MDA levels signify lipid peroxidation, a defining feature of oxidative damage. These results are in line with earlier research that linked oxidative stress to renal damage brought on by cisplatin [23]. It has been demonstrated that the production of ROS, especially hydroxyl radicals, activates apoptotic pathways such as p53 and caspase-3, resulting in renal dysfunction and tubular cell death [24]. The dose-dependent rise in oxidative damage markers and histopathological alterations in our data indicate that oxidative stress is a major mediator of cisplatin-induced nephrotoxicity.

The hepatotoxic effects of cisplatin are characterized by a constellation of histopathological alterations [25]. These include the presence of necrosis, inflammatory infiltrates, and significant disruptions in the overall liver architecture [4]. The accumulation of cisplatin within hepatic tissues exacerbates these effects [26], contributing to the overall severity of liver damage. Similarly, nephrotoxicity arises from the drug's accumulation within renal proximal tubular cells, triggering cellular apoptosis and ultimately compromising renal function [27,2]. This process is intricately linked to

the activation of biochemical pathways associated with oxidative stress [28,9].

The generation of ROS, a central feature of cisplatin-induced toxicity, triggers a cascade of events leading to cellular damage [25,7]. These ROS disrupt cellular function, increasing lipid peroxidation and protein oxidation [29]. Furthermore, ROS activates inflammatory pathways [7,9**],** releasing pro-inflammatory cytokines and chemokines that further exacerbate organ damage [30]. This inflammatory response is coupled with activating apoptotic pathways [25,31], involving key proteins such as p53 and caspase-3, which play crucial roles in initiating and executing programmed cell death [11].

The harmful effects of cisplatin are also significantly influenced by mitochondrial dysfunction [32]. Cisplatin impairs mitochondrial function, which raises ROS generation and lowers ATP synthesis [11]. This mitochondrial dysfunction facilitates the apoptotic and necrotic cell death pathways, which exacerbate organ damage [31]. Significant cellular damage and eventual organ failure result from the disturbance of mitochondrial homeostasis and the initiation of apoptotic pathways [33]. The complexity of cisplatin-induced toxicity is highlighted by the complex interactions among these various mechanistic pathways [2].

The time- and dose-dependent nature of cisplatin-induced hepatorenal toxicity is a critical finding of this study. Higher doses of cisplatin (7 and 10 mg/kg) elicited more severe biochemical and histopathological alterations than the 5 mg/kg dose. Furthermore, the extent of organ damage increased significantly from Day 3 to Day 5, particularly in the 10 mg/kg group. This temporal progression of toxicity highlights the cumulative effects of cisplatin exposure and underscores the importance of monitoring organ function during cisplatin therapy. The findings are consistent with previous reports demonstrating dose- and time-dependent increases in cisplatin-induced organ damage [34,35].

The hepatotoxic and nephrotoxic effects of cisplatin pose significant challenges in clinical oncology, often necessitating dose adjustments or discontinuation of therapy. Our findings highlight the need for protective strategies to mitigate cisplatin-induced organ damage, particularly in high-dose regimens. Antioxidant therapies, such as N-acetylcysteine or vitamin E, have shown promise in preclinical studies for reducing cisplatin-induced oxidative stress and organ toxicity [2,36]. Additionally, targeted drug delivery systems, such as nanoparticles, may help minimize cisplatin accumulation in non-target tissues, thereby reducing its toxic effects [37].

Several limitations should be noted, even though this study offers insightful information about the mechanisms underlying cisplatin-induced hepatorenal toxicity. First, because the study was carried out in a rodent model, the results might not entirely apply to human patients. To confirm these findings, future research should examine the effects of cisplatin in larger animal models or clinical settings. Second, the study focused on short-term toxicity (up to 5 days post-treatment), and the long-term effects of cisplatin exposure remain to be explored. Finally, the study did not investigate potential protective agents or interventions to mitigate cisplatin-induced toxicity. Future research should evaluate the efficacy of antioxidant therapies or targeted drug delivery systems in reducing cisplatin-induced organ damage.

Conclusion

In conclusion, this study demonstrates that cisplatin induces dose- and time-dependent hepatotoxicity and nephrotoxicity in a rat model, mediated primarily through oxidative stress mechanisms. The results emphasize the necessity of protective measures to lessen these negative effects and the crucial part oxidative stress plays in cisplatin-induced organ damage. This study lays the groundwork for further investigations to improve treatment results for cancer patients by clarifying the biochemical and histological changes linked to cisplatin-induced toxicity.

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Declaration of Conflict of Interest

The authors state that there is no conflict of interests

Ethics of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (Approval Number: BUFVTM 05-01-25).

Consent to Participate

All authors contributed to the study conception and design. All authors read and approved of the final manuscript.

Consent for Publication

All authors gave their consent for research publication.

TABLE 1. Liver Function Markers (ALT and AST) at Day 3 and Day 5

Group	ALT (U/L) - Day 3	AST (U/L) - Day 3	ALT (U/L) - Day 5	AST (U/L) - Day 5
Control	38 ± 3.2	74 ± 5.8	39 ± 3.6	76 ± 6.2
Cisplatin 5 mg/kg	52 ± 4.7*	92 ± 8.1*	68 ± 6.0*	108 ± 9.5*
Cisplatin 7 mg/kg	62 ± 5.5**	102 ± 9.0**	82 ± 7.3**	122 ± 10.8**
Cisplatin 10 mg/kg	72 ± 6.4***	117 ± 10.3***	92 ± 8.2***	137± 12.1***

*: Significant **: High significant ***: Very high significant

TABLE 2. Oxidative Stress Markers (Liver MDA and SOD) at Day 3 and Day 5

Group	Liver MDA (nmol/mg tissue) - Day 3	Liver SOD (U/mg tissue) - Day 3	Liver MDA (nmol/mg tissue) - Day 5	Liver SOD (U/mg tissue) - Day 5
Control	0.55 ± 0.05	1.02 ± 0.08	0.56 ± 0.06	1.03 ± 0.09
Cisplatin 5 mg/kg	1.75 ± 0.15*	0.54 ± 0.02*	1.40 ± 0.12*	0.57 ± 0.07*
Cisplatin 7 mg/kg	2.05 ± 0.18**	0.23 ± .01**	2.45 ± 0.21**	0.26 ± 0.01**
Cisplatin 10 mg/kg	2.25 ± 0.20***	0.12± 0.01**	2.75 ± 0.24***	0.01 ± 0.00***

*: Significant **: High significant ***: Very high significant.

TABLE 3. Kidney Function Markers (Urea and Creatinine) at Day 3 and Day 5

Group	Urea (mg/dL) - Day 3	Creatinine (mg/dL) - Day 3	Urea (mg/dL) - Day 5	Creatinine (mg/dL) - Day 5
Control	42 ± 3.5	0.55 ± 0.05	43 ± 3.8	0.56 ± 0.06
Cisplatin 5 mg/kg	85 ± 7.6*	0.95 ± 0.08*	115 ± 10.2*	1.40 ± 0.12*
Cisplatin 7 mg/kg	105 ± 9.3**	1.35 ± 0.12**	145 ± 12.8**	1.75 ± 0.15**
Cisplatin 10 mg/kg	125 ± 11.1***	1.65 ± 0.14***	165 ± 14.6***	1.95 ± 0.17***

*: Significant **: High significant ***: Very high significant

TABLE 4. Oxidative Stress Markers (Kidney MDA and SOD) at Day 3 and Day 5

Group	Kidney MDA (nmol/mg tissue) - Day 3	Kidney SOD (U/mg tissue) - Day 3	Kidney MDA (nmol/mg tissue) - Day 5	Kidney SOD (U/mg tissue) - Day 5
Control	16.5 ± 1.3	1.15 ± 0.09	16.3 ± 1.4	1.16 ± 0.10
Cisplatin 5 mg/kg	30.5 ± 1.1*	0.55 ± 0.06*	25.33 ± 0.9*	0.50 ± 0.07*
Cisplatin 7 mg/kg	40.5 ± 1.0**	0.20 ± 0.08**	35.22 ± 0.7**	0.22 ± 0.01**
Cisplatin 10 mg/kg	52.33 ± 0.8***	0.10 ± 0.00***	48.32 ± 0.6***	0.11 ± 0.04***

*: Significant **: High significant ***: Very high significant

TABLE 5. Histopathological Scoring of Liver Following Cisplatin (Day 3 & Day 5)

Group (D3)	Hepatocellular Damage	Sinusoidal Changes	Inflammatory Infiltration
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cisplatin 5 mg/kg	0.9 ± 0.3	0.7 ± 0.2	0.8 ± 0.2
Cisplatin 7 mg/kg	1.4 ± 0.4	1.3 ± 0.3	1.2 ± 0.2
Cisplatin 10 mg/kg	1.9 ± 0.1	2.0 ± 0.0	1.8 ± 0.2
Group (D5)	**Hepatocellular Damage**	**Sinusoidal Changes**	**Inflammatory Infiltration**
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cisplatin 5 mg/kg	1.1 ± 0.2	1.0 ± 0.2	1.2 ± 0.2
Cisplatin 7 mg/kg	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.2
Cisplatin 10 mg/kg	2.0 ± 0.0	1.9 ± 0.1	1.8 ± 0.1

TABLE 6. Histopathological Scoring of Kidney Following Cisplatin (Day 3 & Day 5)

Group (D3)	Tubular Necrosis	Loss of Brush Border	Cast Formation	Tubular Dilatation
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cisplatin 5 mg/kg	1.3 ± 0.4	1.1 ± 0.3	1.2 ± 0.3	1.0 ± 0.2
Cisplatin 7 mg/kg	2.6 ± 0.4	2.4 ± 0.3	2.5 ± 0.3	2.7 ± 0.4
Cisplatin 10 mg/kg	4.0 ± 0.0	3.8 ± 0.2	3.9 ± 0.1	4.0 ± 0.0
Group (D5)	**Tubular Necrosis**	**Loss of Brush Border**	**Cast Formation**	**Tubular Dilatation**
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cisplatin 5 mg/kg	1.5 ± 0.3	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.3
Cisplatin 7 mg/kg	3.0 ± 0.3	2.8 ± 0.2	2.9 ± 0.3	3.0 ± 0.2
Cisplatin 10 mg/kg	4.0 ± 0.0	3.9 ± 0.1	4.0 ± 0.0	3.8 ± 0.2

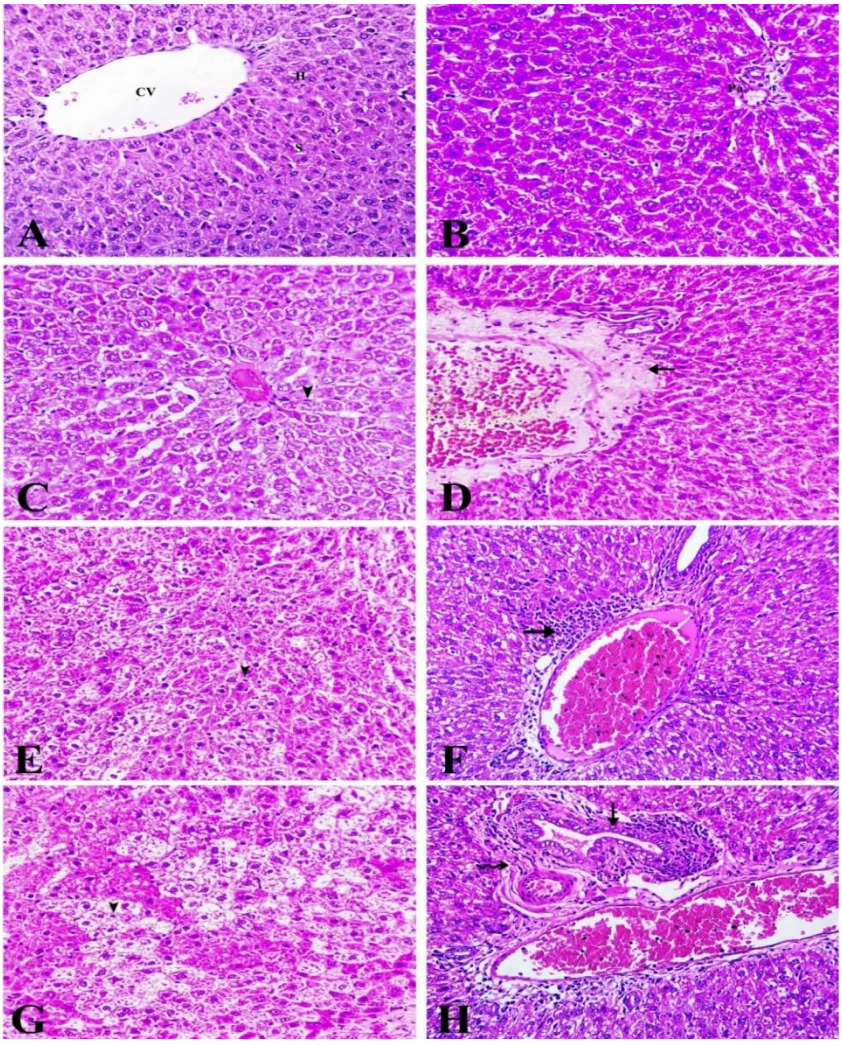

Fig. 1. Representative photomicrographs of liver sections stained with hematoxylin and eosin (x200) from control group (A,B), showing normal liver architecture of central vein (CV), blood sinusoids (S) with intact hepatocyte (H) organization with normal portal areas (pa). Rat treated with 5 mg/kg (C, D), showing hydropic changes and cytoplasmic vacuolization (arrowhead), moderate portal inflammation (arrow). Rat treated with 7 mg/kg (E, F), showing more pronounced hepatocellular necrosis (arrowhead), with extensive inflammatory infiltrates (arrow) and mild fibrosis in portal regions. Rat treated with 10 mg/kg (G, H) group, showing significant loss of hepatic architecture and marked hepatocellular necrosis (arrowhead), bile duct hyperplasia (arrow), and significant portal fibrosis (zigzag arrow).

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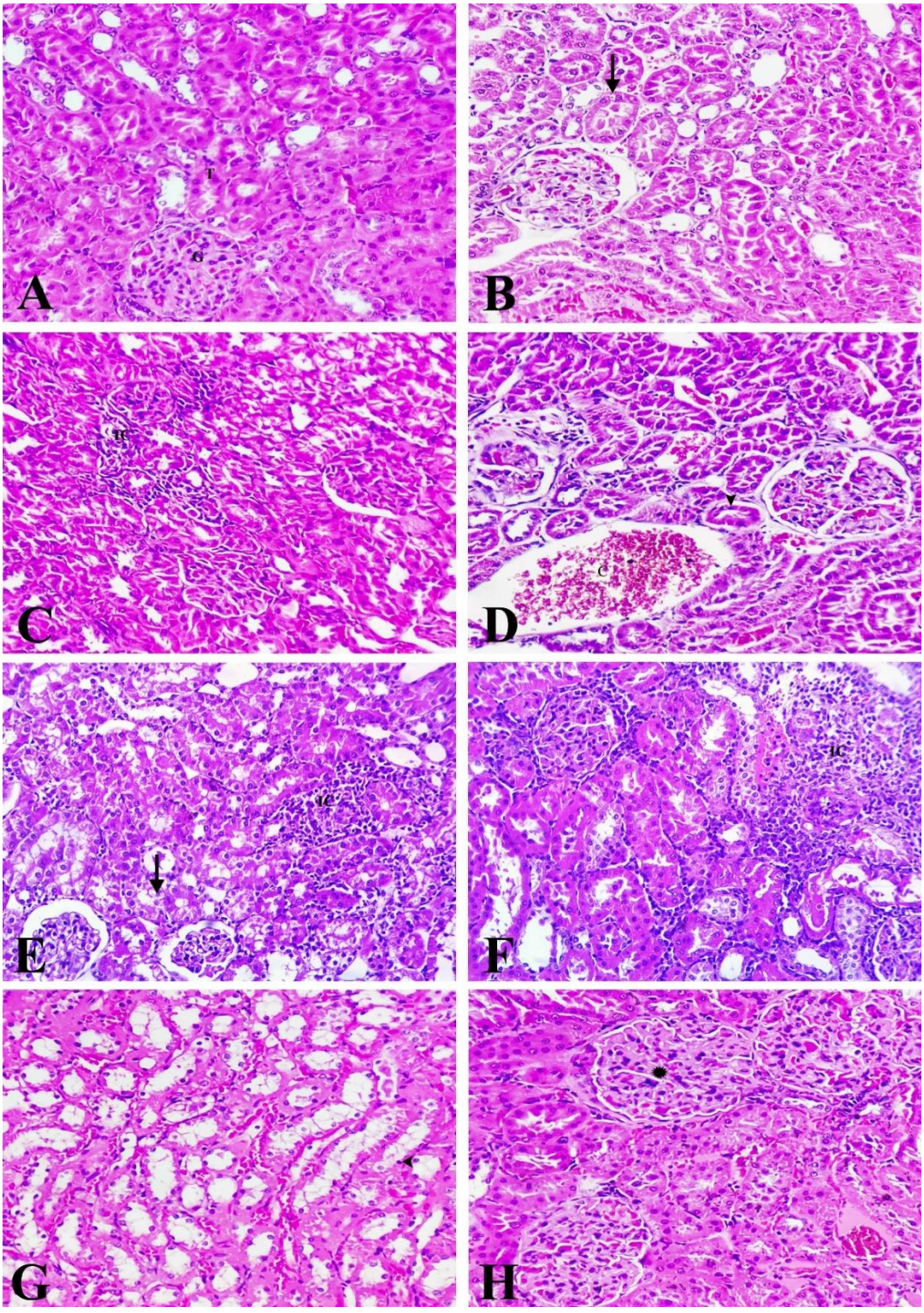

Fig. 2. Representative photomicrographs of kidney sections stained with hematoxylin and eosin (x200) from control group (A), showing normal glomeruli (G) and well-preserved tubular architecture (T). Rat treated with 5 mg/kg (B, C), showing hydropic changes in proximal tubular cells (arrow), with moderate inflammatory cell (IC) infiltrate, primarily consisting of lymphocytes. Rat treated with 7 mg/kg (D, E), showing congestion (C) of renal blood vessels, more pronounced tubular necrosis (arrowhead), especially in proximal convoluted tubules, with extensive cytoplasmic vacuolation (arrow). Also noted marked interstitial inflammation (IC). Rat treated with 10 mg/kg (F, G, H), showing extensive interstitial infiltration (IC) with severe tubular necrosis (arrowhead). Also noted glomerular changes potentially including hypercellularity (asterisk) and initial sclerosis.

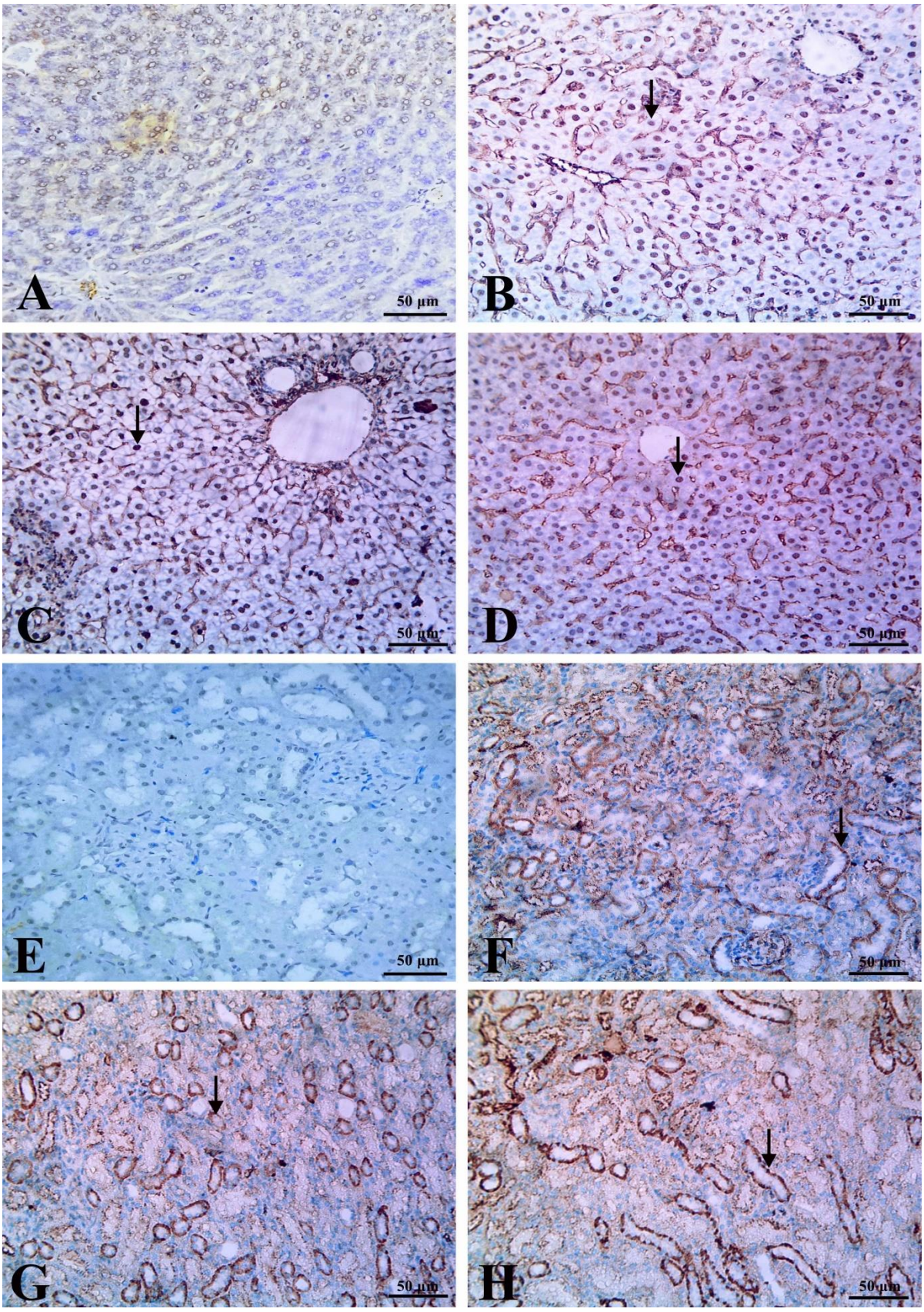

Fig. 3. Representative photomicrographs of liver and kidney sections of different experimental groups, showing immunohistochemical localization of caspase 3, black arrows: nuclear immune expression in hepatocytes and renal tubular cells. (A, E) Control group. (B, F) Cisplatin 5 mg/kg group. (C, G) Cisplatin 7 mg/kg group, (D, H) Cisplatin 10 mg/kg group. (scale bar = 50 µm)

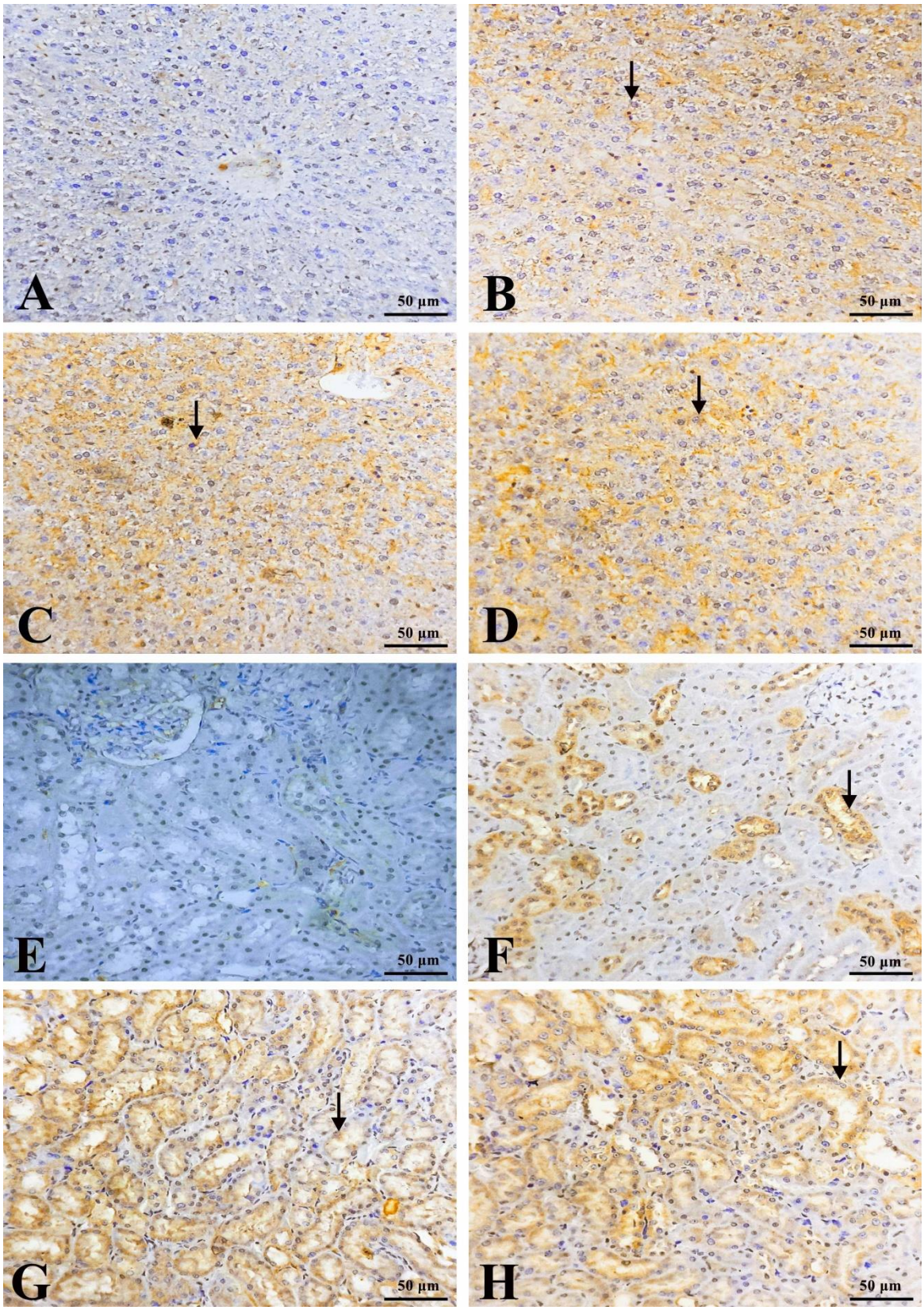

Fig. 4. Representative photomicrographs of liver and kidney sections of different experimental groups showing immunohistochemical localization of tumor necrosis factor (TNF), black arrows: cytoplasmic immune expression in hepatocytes and renal tubular cells. (A, E) Control group. (B, F) Cisplatin 5 mg/kg group. (C, G) Cisplatin 7 mg/kg group, (D, H) Cisplatin 10 mg/kg group. (scale bar = 50 µm).

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تأثير السيسبلاتين في إحداث الإجهاد التأكسدى وتضرر الكبد والكلية: دراسة سمية باثولوجية تجريبية على نماذج الفئران.

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الملخص

يُعَدُّ السيسبلاتين دواءً كيميائياً أساسياً في علاج الأورام، لكن سميّته الجرعوية على الكبد والكلى تُحدّ من استخدامه. قامت هذه الدراسة بتقييم التلف الكبدي والكلوي الناجم عن السيسبلاتين في فئران مخبرية باستخدام جرعات 5، 7، و10 ملغ/كغ، مع تحليل التغيرات الكيميائية الحيوية وإجهاد الأكسدة والنسيجية بعد 3 و5 أيام من الحقن. أظهرت النتائج ارتفاعاً جرعوياً وزمنياً في إنزيمات الكبد عند الجرعة 10 ملغ/كغ بحلول اليوم الخامس، مع تأكيد النتائج النسيجية على نخر خلايا الكبد والتهابات وتليف بابي. في الكبد، ارتفعت مؤشرات أكسدة الدهون مع استجابة تعويضية غير كافية من الإنزيم المضاد للأكسدة SOD. في المقابل، أظهرت الكلى ارتفاعاً في الكرياتينين واليوريا مع انخفاض حاد في نشاط SOD الكلوي وزيادة MDA ، مما يشير إلى فشل الدفاعات المضادة للأكسدة. كشفت الفحوص النسيجية تفاقماً في النخر الأنبوبي الكلوي والتلف الكبدي مع زيادة الجرعة والزمن، بينما أظهر التحليل المناعي زيادة تعبير بروتينات caspase-3 وTNF-α، مما يؤكد دور الإجهاد التأكسدي والالتهاب في تلف الأعضاء. وقد اوضحت النتائج اختلافاً في استجابة الأعضاء للإجهاد التأكسدي، حيث يحاول الكبد تعويض الضرر مؤقتاً، بينما تفشل الكلى في ذلك، مما يستدعي مراقبة دقيقة أثناء العلاج واعتماد استراتيجيات وقائية مثل مضادات الأكسدة أو أنظمة توصيل دوائية مستهدفة لتقليل السمية دون التأثير على الفعالية العلاجية. تُقدِّم هذه الدراسة رؤىً حاسمة حول التفاعل بين الإجهاد التأكسدي وتلف الأعضاء، مما يُعزّز تطوير أساليب لتحسين سلامة العلاج الكيميائي سريرياً.

الكلمات الدالة: السيسبلاتين، التغيرات الكيميائية الحيوية، الإجهاد التأكسدي، الضرر الكبدي الكلوي، االفئران.