Ranolazine reduces oxidative damage and improves kidney function in cisplatin-induced nephrotoxicity

Lokman Hekim Tanriverdi[1], Hatice Goncu[1], Mehmet Gunata[1], Ali Beytur[1], Nigar Vardi[1], Yilmaz Cigremis[1], and Hakan Parlakpinar[1]

[1]Inonu Universitesi Karaciger Nakli Enstitusu

May 19, 2023

Abstract

Cisplatin (CIS)-induced nephrotoxicity is associated with oxidative stress, apoptosis, inflammation, and fibrogenesis. In this study, we investigated the therapeutic effects of ranolazine (RAN), a current antianginal drug known to experimentally reduce oxidative damage in CIS-induced nephrotoxicity. We randomly assigned thirty-two Sprague Dawley rats to one of four groups (Control, CIS, CIS+RAN, and RAN+CIS). We evaluated kidney function parameters including blood urea nitrogen (BUN), creatinine (Cre), electrolytes, and albumin, as well as tissue biochemical parameters including malondialdehyde (MDA) and antioxidant enzymes. Histopathological parameters were also assessed. We observed a significant increase in BUN and Cre values in the CIS group compared to the control group ($p<0.05$). However, there was a significant decrease in BUN values ($p<0.05$) in the CIS+RAN and RAN+CIS groups compared to the CIS group. In contrast, the decrease in Cre values did not reach statistical significance ($p>0.05$), and serum electrolytes were comparable among groups. Furthermore, a statistically significant increase in albumin levels was observed in the CIS+RAN group compared to the CIS group ($p<0.05$). MDA levels were significantly decreased in the CIS+RAN group compared to the CIS group, indicating the antioxidant activity of RAN ($p<0.05$). Histopathological analysis revealed that necrosis and dilatation in epithelial cells of cortical and medullary tubules were more prominent in the CIS group ($p<0.0001$). However, in the RAN+CIS group, the histopathological changes observed in the CIS group were found to be significantly reduced ($p<0.0001$). Degenerative changes in tubules were observed in the CIS+RAN group ($p>0.05$). Our findings suggest that the beneficial effects of RAN on CIS-induced nephrotoxicity may be related to its antioxidant activity.

INTRODUCTION

Cisplatin (CIS) is an antineoplastic agent used to treat various types of cancer, including testicular, ovarian, bladder, and lung cancer [1]. However, its use is limited due to its cumulative and dose-dependent toxic effects, such as nephrotoxicity, neurotoxicity, and ototoxicity [2]. CIS is known to accumulate significantly in the kidneys, particularly in the proximal tubules, with the S3 segment being the most affected [3]. The cellular pathology of nephrotoxicity is mediated by oxidative stress, apoptosis, inflammation, and fibrogenesis [4]. CIS-mediated oxidative stress in renal tubules is initiated by reactive oxygen species (ROS) and intracellular Ca^{2+}elevation [5].

Ranolazine (RAN) is an antianginal drug that has been studied for several purposes [6]. RAN prevents the increase in Na^{+} by blocking late Na^{+} channels and the accumulation of cellular Ca^{2+} through Na/Ca^{2+} exchange (NCX) and restores mitochondrial function, thereby exerting an antioxidant effect by preventing the formation of ROS [7, 8]. It has been shown that oxidative stress triggers the late Na+ channel, and the accumulation of Na+ and Ca2+ is the main mechanism in organ damage [9]. Thus, RAN can counteract the harmful effects of oxidative stress [10].

Currently, preventive strategies, such as monitoring kidney function before starting nephrotoxic drugs, close

patient follow-up, and fluid administration, are recommended to prevent CIS-induced nephrotoxicity [11]. However, CIS nephrotoxicity remains a significant clinical problem that requires effective solutions. CIS and RAN have opposing effects mediated by common mechanisms, such as oxidant-antioxidant enzyme levels. Therefore, we aimed to evaluate the protective and therapeutic effects of RAN against CIS-induced nephrotoxicity in terms of oxidative stress, renal function markers, and histopathological parameters.

MATERIAL-METHOD

The study used 32 male Sprague Dawley rats weighing between 250-300 g and aged 4-6 months. The rats were obtained from the İnönü University Experimental Animals Production and Research Center and were housed in a temperature (21±2°C) and humidity (60±5%) controlled room with a 12:12 h light: dark cycle. The rats were fed a standard chow pellet diet and had access to pelleted diet and drinking water ad libitum. The study protocol was approved by the Ethics Committee on Animal Research under the Faculty of Medicine at İnönü University, Malatya, Türkiye (protocol no: 2019/A-21). All experimental procedures were carried out by investigators that blinded the experimental groups and in accordance with the ARRIVE guidelines [12].

A simple randomization technique was used to assign animals to the experimental groups (n=8 for each group):

Control group: A single dose of 2 mL saline was intraperitoneally (i.p.) administered, and the experiment was terminated 72 hours later.

CIS group: A single dose of 8 mg/kg CIS was i.p. administered, and the experiment was terminated 72 hours later.

RAN+CIS group: The rats were given 50 mg/kg RAN orally for 5 days, and a single dose of 8 mg/kg CIS was administered i.p. on the 3rd day. The experiment was terminated on the 6th day.

CIS+RAN group: A single dose of 8 mg/kg CIS was administered i.p. at baseline, and the rats were given 50 mg/kg RAN orally for 5 days starting from the 3rd day. The experiment was terminated on the 6th day.

Rat weights were recorded prior to anesthesia. General anesthesia was induced with 5 mg/kg xylazine and 75 mg/kg ketamine i.p. confirmed with a finger pinching response. Intracardiac blood samples were collected to measure biochemical parameters such as blood urea nitrogen (BUN), creatinine (Cre), albumin, Na^+, Cl^-, K^+, and Ca^{2+}. The blood was centrifuged at 3000 rpm for 10 minutes, and the serum was separated for spectrophotometric measurement of biochemical markers in the Architect C16000 device (Abbott, Chicago, IL, USA) following the manufacturer's instructions. The right kidneys were removed, weighed on a high-precision laboratory scale, and stored at -80 degrees for analysis of tissue biochemistry parameters such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA). The capsule of the left kidney was carefully peeled, washed with saline, and kept at room temperature in 10% formaldehyde-containing boxes for histopathological analysis.

CISPLATIN DBL 100 mg/ 100 mI Injectable Solution (ORNA, Istanbul) and Latixa 375 Mg Extended-Release Tablet (Menarini, Istanbul) were used as the chemical preparations.

Histological Analysis

Following tissue follow-up procedures, 4-5 μm thick sections were obtained from the paraffin blocks. The sections were subjected to hematoxylin-eosin (H&E) staining to examine general morphological structures.

Kidney sections were evaluated for tubular degeneration (cast formation, tubular necrosis, and dilatation) in the cortical and medullary areas. Ten randomly selected areas were assessed based on the degree of histological changes and scored as follows: 0 for no change, 1 for mild, 2 for moderate, and 3 for severe changes [13].

The analyses were performed using the Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) with the Leica DFC-280 research microscope.

Biochemical Analysis

Protein determination in the tissue was performed by Biuret protein analysis using bovine serum albumin as a standard [14].

MDA, an indicator of lipid peroxidation, was assessed according to the method described by Uchiyama and Mihara [15]. The rat kidney sample was homogenized in a 1.15% KCl solution on ice for 1 minute at 15000 rpm to obtain a 10% homogenate. This homogenate was used directly in the MDA analysis, and the results were expressed as nmol/gram tissue.

GSH was determined according to Ellman's method [16]. The rat kidney sample was homogenized on ice to form a 10% homogenate at 15000 rpm for 1-2 minutes. The homogenate was then centrifuged at 3000 rpm, +4 degrees, for 15 minutes. The resulting supernatant TCA solution was added, mixed, and centrifuged again, making the sample ready for GSH analysis, and the results were expressed as nmol/g tissue.

Tissue SOD activity was measured according to the method described by Sun et al. [17]. The rat kidney sample was homogenized on ice for 1 minute at 15000 rpm to obtain a 10% homogenate. The homogenate was then centrifuged at 10000 rpm for 20 minutes. A 3:5 ratio of chloroform/ethanol (3 parts chloroform to 5 units of ethanol) was added to the supernatant, and the samples were centrifuged again at 5000 rpm at +4 degrees for 20 minutes. The top clear white chloroform phase was carefully taken with a pipette and used in Cu/Zn-SOD analysis. The enzyme activity was expressed as U/g protein.

Tissue CAT activity was measured according to Luck's method [18]. The rat kidney sample was homogenized on ice for 1 minute at 15000 rpm to obtain a 10% homogenate. The homogenate was then centrifuged at 10000 rpm for 20 minutes and used for the supernatant CAT analysis. The results were presented as K/g protein.

Statistical Analysis

The statistical analysis was conducted using the statistical software developed by the Department of Biostatistics and Medical Informatics at İnönü University, Faculty of Medicine, Malatya, Türkiye [19]. Normality tests showed that the measurable variables in all groups did not follow a normal distribution. Therefore, the Kruskal-Wallis analysis of variance, a non-parametric test, was used to compare the groups for all variables. The Mann-Whitney-U test with Bonferroni correction was used to compare between the two groups. Statistical significance was considered at $p<0.05$. The data were presented as mean with 95% confidence interval (lower-upper bound).

RESULTS

Death and Weight

During the course of the experiment, it was observed that three rats expired within the CIS+RAN group. In order to achieve uniformity in sample size across all groups and to obtain sufficient data for statistical analysis, three additional rats were included in the same group. Upon completion of the experiment, the weights of the rats and their respective kidneys were measured. Notably, no statistically significant differences were observed among the groups ($p>0.05$) (Figure 1).

Kidney Function Tests

-BUN and Cre

Upon further examination of the results, it was observed that there was a significant increase in BUN and Cre values among all groups that were administered with CIS (CIS, CIS+RAN, RAN+CIS), as compared to the control group ($p<0.05$). Furthermore, a significant decrease in BUN values ($p<0.05$) was observed in the CIS group, as compared to the CIS+RAN group. However, no significant differences in Cre values were noted among the groups ($p>0.05$) (Figure 2).

-Electrolytes and Albumin

The electrolytes Na^+, Cl^-, K^+, and Ca^{2+} were measured to evaluate the filtration capacity of the kidneys. However, neither the administration of CIS nor therapeutic RAN produced any significant differences among the groups ($p>0.05$).

Serum albumin values in the CIS group showed a non-significant decrease as compared to the control group ($p>0.05$). Conversely, albumin levels were significantly increased in the CIS+RAN group, as compared to the CIS group ($p<0.05$) (Figure 2).

Histopathological Findings

The renal tissue in the control group exhibited a normal histological appearance (Figure 3A). In contrast, the CIS group displayed cortical and medullary tubular degenerative changes, necrosis of epithelial cells in cortical tubules, and dilatation of medullary tubules, along with caste formation observed in the lumen of some tubules (Figure 3B). Notably, the differences between the control and CIS groups were found to be statistically significant ($p<0.0001$).

In the RAN+CIS group, the observed histopathological changes were significantly reduced as compared to the CIS group ($p<0.0001$) (Figure 3C). However, degenerative changes in the tubules persisted, and the severity of tubular degeneration, especially tubular dilatation, was significantly higher in the CIS+RAN group than in the CIS group ($p<0.0001$) (Figure 3D). The histopathological evaluation results of the groups are presented in Figure 3E.

Tissue Biochemistry

MDA, a biomarker that indicates oxidative stress as a lipid peroxidation product, showed a significant increase in the CIS group as compared to the Control group ($p<0.05$). Intriguingly, MDA levels were significantly higher in the RAN+CIS group than in the CIS and CIS+RAN groups ($p<0.05$) (Figure 4).

SOD, CAT, and GSH are antioxidant enzymes that work to prevent damage caused by ROS and take part in different enzymatic steps to render these radicals harmless. SOD levels were decreased in the CIS and RAN+CIS groups, as compared to the Control group, but increased nonsignificantly in the CIS+RAN group ($p>0.05$). CAT and GSH values were similar among groups ($p>0.05$) (Figure 4).

DISCUSSION

The kidney plays vital roles in maintaining homeostasis, including the regulation of water and electrolyte balance and the elimination of endogenous and exogenous metabolites [20]. Despite the known nephrotoxicity of CIS since its introduction to the market many years ago, it continues to be widely prescribed, despite efforts to find less toxic but equally effective alternatives [21]. CIS-induced nephrotoxicity is associated with oxidative stress, which plays a significant role in the pathogenesis of the condition [1, 4].

Numerous studies have demonstrated that CIS-induced nephrotoxicity causes a decrease in kidney function, with an increase in serum creatinine and BUN levels. For instance, in a clinical trial involving 400 patients, serum creatinine reached the upper limit of normal during follow-up in 42% of patients after CIS administration, which was defined as nephrotoxicity [22]. Consistent with the literature, our study showed that BUN and creatinine levels increased in the CIS group but remained similar in the other groups [23]. Furthermore, a study showed that prophylactic administration of RAN preserved BUN and creatinine values in patients with contrast-induced kidney damage. The protective effect of RAN on renal function can be attributed to its modulation of intracellular Ca2+ homeostasis, oxidative stress, and suppression of apoptosis [24].

Research findings indicate that CIS irreversibly binds to albumin, a plasma protein [25]. Consequently, predicting the safe drug range in tissues based on plasma CIS levels is challenging. Burns et al. proposed a clinical risk score algorithm that incorporated patient age, dosage, hypertension, and albumin levels to forecast CIS nephrotoxicity [26]. Another study found a correlation between low pre-treatment albumin levels and neutropenia after CIS treatment [27]. However, albumin was regarded as a risk parameter rather than a marker of nephrotoxicity. In our investigation, no significant changes in albumin levels were observed.

The renal tubules regulate the serum levels of electrolytes by reabsorbing them. In an experimental study on CIS nephrotoxicity, renal losses were believed to be the cause of hypomagnesemia, hypocalcemia, and hypokalemia [28]. Goren et al. noted that electrolyte imbalances may be influenced by the cumulative dose of CIS [29]. It is possible that the study design did not induce cumulative dose toxicity, which may explain why other electrolytes did not change as expected. Additionally, Oronsky et al. conducted an extensive review of CIS and electrolyte disorders [30]. The variability in electrolyte levels may be due to their different mechanisms and presence in various tissues (e.g., Ca2+ is influenced by parathormone and vitamin D, and losses occur from bone to kidney and intestine) and measurement methods. A clinical study demonstrated a significant reduction in the incidence of nephrotoxicity with the administration of Mg, Ca, and K supplements during CIS treatment [31]. Moreover, in their study, Ma et al. evaluated intracellular Na flow by confocal microscopy, which contributed to the renoprotective effect of RAN due to its impact on the late Na^+ channel [24].

The formation and removal of ROS must be balanced to prevent oxidative stress. Disturbance of this balance can lead to oxidative stress, emphasizing the importance of measuring it with appropriate biomarkers and treating it with suitable antioxidant substances [32]. MDA is among the most commonly used markers of oxidative stress, as it indicates lipid peroxidation. Non-enzymatic elements of the antioxidant system that help reduce ROS levels include GSH, vitamin C, and E, while enzymatic antioxidants comprise SOD, CAT, and GSH [33-35].

Yu et al. investigated the effectiveness of Hesperetin against CIS nephrotoxicity, utilizing techniques such as Western blot analysis, fluorescence staining, and tissue biochemistry similar to those in our study. Additionally, inflammation and apoptosis were responsible for CIS-induced renal damage [36]. Our findings were consistent with their results, as MDA levels were among the increased oxidant parameters in CIS-administered rats [37, 38]. Furthermore, a study examining the antidiabetic effect of RAN demonstrated a significant decrease in MDA levels in pancreatic tissue [39]. However, in another study, RAN did not cause changes in MDA levels in rat hearts [40].

Superoxide anion, hydrogen peroxide, and hydroxyl radical are substances that play a significant role in CIS-induced organ damage [41]. SOD, CAT, and GSH are enzymatic antioxidants that convert these harmful substances into harmless forms through different enzymatic steps [33]. In a study by Ehsan et al., Casticin was used as an antioxidant against CIS-induced renal toxicity. They found that Casticin application increased the levels of SOD, CAT, and GSH, which were reduced by CIS. Additionally, the levels of thiobarbituric acid reactive substances, hydrogen peroxide, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin that they examined also supported their hypothesis [42]. Similarly, in a lung malignancy study where the antioxidant effect of RAN at doses of 50 and 100 mg was measured using MDA, SOD, CAT, and GSH, a significant decrease was observed in MDA values, and an increase in the antioxidant parameters SOD, CAT, and GSH compared to the malignancy group. These findings suggested the protection of RAN through a dose-dependent antioxidant mechanism, as reported in the literature [43]. Based on these results, we believe that further studies could demonstrate the same antioxidant effect on the kidney.

ROS can disrupt mitochondrial permeability and lead to cell necrosis and apoptosis. In the context of acute kidney injury caused by ischemia and nephrotoxic agents, ROS is considered an early pathological factor [44]. In our study, we observed necrosis in the cortical tubule epithelium and dilatation in the medullary tubules, accompanied by caste formation in some tubules, upon histopathological examination of the kidney tissue at the end of the study period. These findings are consistent with the results of a study by Parlakpinar et al. where necrosis and tubular dilatation were also observed in the tubule epithelium [45]. RAN was administered as a pre-treatment against contrast-induced kidney damage in a study where histopathological changes were found to be improved [24]. In our study, we observed that there was no significant difference in damage between the CIS+RAN group and the CIS group, with a nonsignificant increase in the degree of dilatation observed in the former group.

CONCLUSION

In sum, our study confirmed the nephrotoxicity of CIS through analysis of various biochemical and histopathological parameters, which suggested an imbalance in oxidant and antioxidant levels leading to oxidative stress. While the effects of RAN treatment/prophylaxis were not consistently observed across all parameters, the improvement in kidney function highlights the need for further and more comprehensive studies on the potential benefits of RAN.

Acknowledgments

This work was supported by the Scientific and Technological Research Council of Turkey under Grant [2209-A/1919B011900466].

Declaration of Interest Statement

The authors report there are no competing interests to declare.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, [AB], upon reasonable request.

References

[1] Ghosh S. Cisplatin: The first metal based anticancer drug. Bioorg Chem. (2019) **88** , 102925.

[2] Rani N., Bharti S., Tomar A., Dinda A.K., Arya D.S. & Bhatia J. Inhibition of PARP activation by enalapril is crucial for its renoprotective effect in cisplatin-induced nephrotoxicity in rats. Free Radic Res. (2016) **50** , 1226-36.

[3] Kuhlmann M.K., Burkhardt G. & Kohler H. Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application. Nephrol Dial Transplant. (1997) **12** , 2478-80.

[4] Manohar S. & Leung N. Cisplatin nephrotoxicity: a review of the literature. J Nephrol. (2018) **31** , 15-25.

[5] Kawai Y., Nakao T., Kunimura N., Kohda Y. & Gemba M. Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. J Pharmacol Sci. (2006)**100** , 65-72.

[6] Uran C. Through the heart and beyond: a review on ranolazine. Monaldi Archives for Chest Disease. (2021).

[7] Aldakkak M., Camara A.K., Heisner J.S., Yang M. & Stowe D.F. Ranolazine reduces Ca2+ overload and oxidative stress and improves mitochondrial integrity to protect against ischemia reperfusion injury in isolated hearts. Pharmacol Res. (2011) **64** , 381-92.

[8] Özdemir M. Ranolazin'in antianginal etki mekanizması. Turk Kardiyol Dern Ars. (2016) **44** , 8-12.

[9] Song Y., Shryock J.C., Wagner S., Maier L.S. & Belardinelli L. Blocking late sodium current reduces hydrogen peroxide-induced arrhythmogenic activity and contractile dysfunction. J Pharmacol Exp Ther. (2006) **318** , 214-22.

[10] Zhang X.Q., Yamada S. & Barry W.H. Ranolazine inhibits an oxidative stress-induced increase in myocyte sodium and calcium loading during simulated-demand ischemia. J Cardiovasc Pharmacol. (2008)**51** , 443-9.

[11] Duffy E.A., Fitzgerald W., Boyle K. & Rohatgi R. Nephrotoxicity: Evidence in Patients Receiving Cisplatin Therapy. Clin J Oncol Nurs. (2018) **22** , 175-83.

[12] Colak C. & Parlakpinar H. Animals in research: reporting In vivo experiments: ARRIVE guidelines-review. (2012).

[13] Tasdemir C., Tasdemir S., Vardi N., Ates B., Parlakpinar H., Kati B., et al. Protective effect of infliximab on ischemia/reperfusion-induced damage in rat kidney. Ren Fail. (2012)**34** , 1144-9.

[14] Hiller A., Greif R.L. & Beckman W.W. Determination of protein in urine by the biuret method. J Biol Chem. (1948) **176** , 1421-9.

[15] Uchiyama M. & Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical biochemistry. (1978) **86** , 271-8.

[16] Ellman G.L. Tissue sulfhydryl groups. Arch Biochem Biophys. (1959) **82** , 70-7.

[17] Sun Y., Oberley L.W. & Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. (1988) **34** , 497-500.

[18] Cigremis Y., Akgoz M., Ozen H., Karaman M., Kart A., Gecer M., et al. Resveratrol ameliorates cisplatin-induced oxidative injury in New Zealand rabbits. Can J Physiol Pharmacol. (2015) **93** , 727-35.

[19] Yaşar Ş., Arslan A., Colak C. & Yoloğlu S. A developed interactive web application for statistical analysis: statistical analysis software. Middle Black Sea Journal of Health Science. (2020)**6** , 227-39.

[20] Davenport A. The brain and the kidney–organ cross talk and interactions. Blood Purif. (2008) **26** , 526-36.

[21] Aldossary S.A. Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. Biomedical and Pharmacology Journal. (2019) **12** , 7-15.

[22] De Jongh F., Van Veen R., Veltman S., de Wit R., Van der Burg M., Van den Bent M., et al. Weekly high-dose cisplatin is a feasible treatment option: analysis on prognostic factors for toxicity in 400 patients. British journal of cancer. (2003) **88** , 1199-206.

[23] Hosoda A., Matsumoto Y., Toriyama Y., Tsuji T., Yoshida Y., Masamichi S., et al. Telmisartan Exacerbates Cisplatin-Induced Nephrotoxicity in a Mouse Model. Biol Pharm Bull. (2020) **43** , 1331-7.

[24] Ma C., Chen T., Ti Y., Yang Y., Qi Y., Zhang C., et al. Ranolazine alleviates contrast-associated acute kidney injury through modulation of calcium independent oxidative stress and apoptosis. Life Sci. (2021) **267** , 118920.

[25] Park C.R., Kim H.Y., Song M.G., Lee Y.S., Youn H., Chung J.K., et al. Efficacy and Safety of Human Serum Albumin-Cisplatin Complex in U87MG Xenograft Mouse Models. Int J Mol Sci. (2020) **21** .

[26] Burns C.V., Edwin S.B., Szpunar S. & Forman J. Cisplatin-induced nephrotoxicity in an outpatient setting. Pharmacotherapy. (2021) **41** , 184-90.

[27] Nakazawa Y., Kageyama A., Kitamura M., Mitsumori N. & Kawakubo T. Prediction of Severe Cisplatin-Induced Neutropenia Using Serum Albumin Concentration: A Retrospective Study. (2020).

[28] Abdel-Gayoum A.A. & Ahmida M.H.S. Changes in the serum, liver, and renal cortical lipids and electrolytesin rabbits with cisplatin-induced nephrotoxicity. Turkish journal of medical sciences. (2017) **47** , 1019-27.

[29] Goren M.P. Cisplatin nephrotoxicity affects magnesium and calcium metabolism. Med Pediatr Oncol. (2003) **41** , 186-9.

[30] Oronsky B., Caroen S., Oronsky A., Dobalian V.E., Oronsky N., Lybeck M., et al. Electrolyte disorders with platinum-based chemotherapy: mechanisms, manifestations and management. Cancer Chemother Pharmacol. (2017) **80** , 895-907.

[31] Minzi O.M.S., Lyimo T.E., Furia F.F., Marealle A.I., Kilonzi M., Bwire G.M., et al. Electrolytes supplementation can decrease the risk of nephrotoxicity in patients with solid tumors undergoing chemotherapy with cisplatin. BMC Pharmacol Toxicol. (2020) **21** , 69.

[32] Parlakpinar H., Ozhan O., Ermis N., Vardi N., Cigremis Y., Tanriverdi L.H., et al. Acute and Subacute Effects of Low Versus High Doses of Standardized Panax ginseng Extract on the Heart: An Experimental

Study. Cardiovasc Toxicol. (2019) **19** , 306-20.

[33] Chirino Y.I. & Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. Exp Toxicol Pathol. (2009) **61** , 223-42.

[34] Marrocco I., Altieri F. & Peluso I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. Oxid Med Cell Longev. (2017) **2017** , 6501046.

[35] Zhou H., Kato A., Miyaji T., Yasuda H., Fujigaki Y., Yamamoto T., et al. Urinary marker for oxidative stress in kidneys in cisplatin-induced acute renal failure in rats. Nephrol Dial Transplant. (2006) **21** , 616-23.

[36] Yu X., Meng X., Xu M., Zhang X., Zhang Y., Ding G., et al. Celastrol ameliorates cisplatin nephrotoxicity by inhibiting NF-kappaB and improving mitochondrial function. EBioMedicine. (2018) **36** , 266-80.

[37] An J.H., Li C.Y., Chen C.Y., Wu J.B. & Shen H. Raloxifene Protects Cisplatin-Induced Renal Injury in Mice via Inhibiting Oxidative Stress. Onco Targets Ther. (2021) **14** , 4879-90.

[38] Chen X., Wei W., Li Y., Huang J. & Ci X. Hesperetin relieves cisplatin-induced acute kidney injury by mitigating oxidative stress, inflammation and apoptosis. Chem Biol Interact. (2019) **308** , 269-78.

[39] Elkholy S.E. The Possible Antidiabetic Effects of Ranolazine Versus Gliclazide In HFD/STZ-Induced Type 2 Diabetes In Male Albino Rats. Medicine Updates. (2020) **1** , 9-28.

[40] Matsumura H., Hara A., Hashizume H., Maruyama K. & Abiko Y. Protective effects of ranolazine, a novel anti-ischemic drug, on the hydrogen peroxide-induced derangements in isolated, perfused rat heart: comparison with dichloroacetate. Jpn J Pharmacol. (1998) **77** , 31-9.

[41] Karakoc H.T., Altintas R., Parlakpinar H., Polat A., Samdanci E., Sagir M., et al. Protective Effects of Molsidomine Against Cisplatin-Induced Nephrotoxicity. Adv Clin Exp Med. (2015) **24** , 585-93.

[42] Ehsan N., Ijaz M.U., Ashraf A., Sarwar S., Samad A., Afzal G., et al. Mitigation of cisplatin induced nephrotoxicity by casticin in male albino rats. Braz J Biol. (2021) **83** , e243438.

[43] ullah Baig M.N., Alvi S.B., Alvala M., Sama V., Padmavathi Y., Ramadevi P., et al. Ranolazine as a Protective Agent Against Lung Cancer: A Translational Approach. Asian Journal of Pharmaceutical and Health Sciences. (2020) **10** .

[44] Su H., Wan C., Song A., Qiu Y., Xiong W. & Zhang C. Oxidative Stress and Renal Fibrosis: Mechanisms and Therapies. Adv Exp Med Biol. (2019) **1165** , 585-604.

[45] Parlakpinar H., Sahna E., Ozer M.K., Ozugurlu F., Vardi N. & Acet A. Physiological and pharmacological concentrations of melatonin protect against cisplatin-induced acute renal injury. J Pineal Res. (2002) **33** , 161-6.

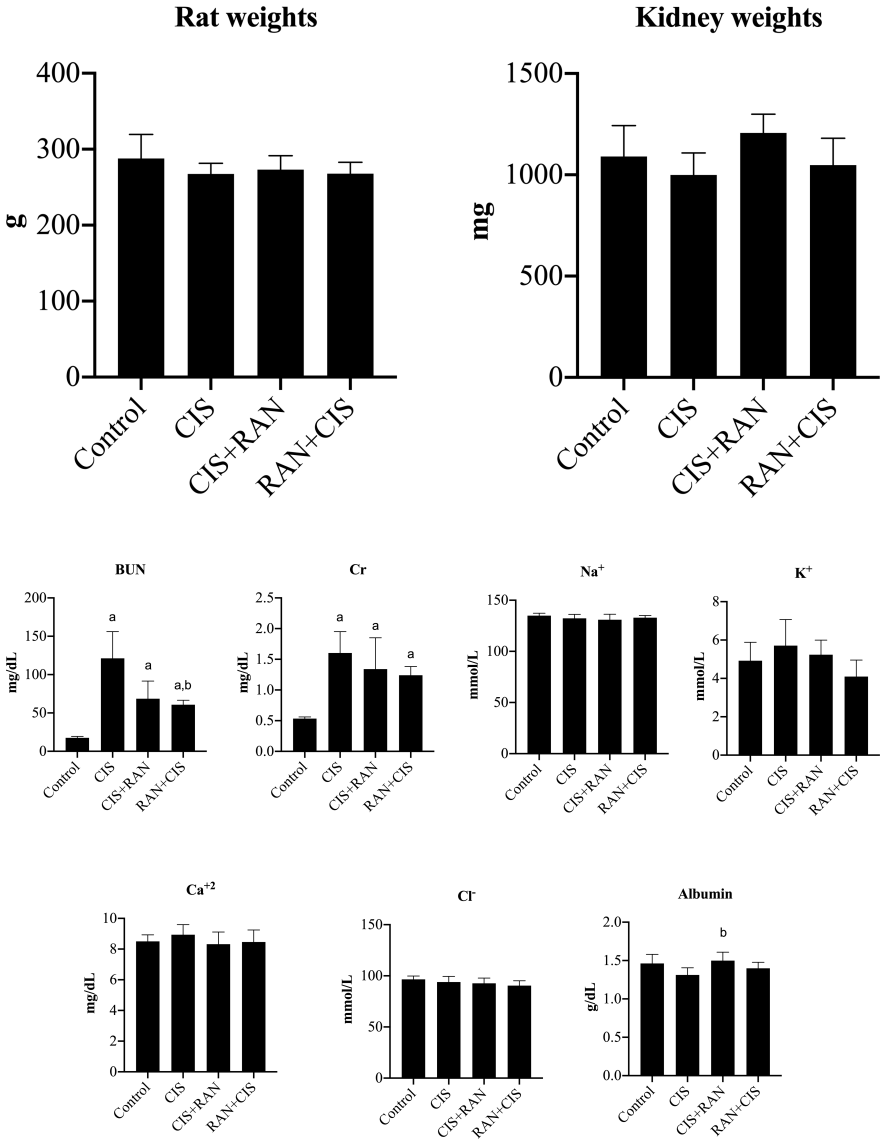
Figure Legends

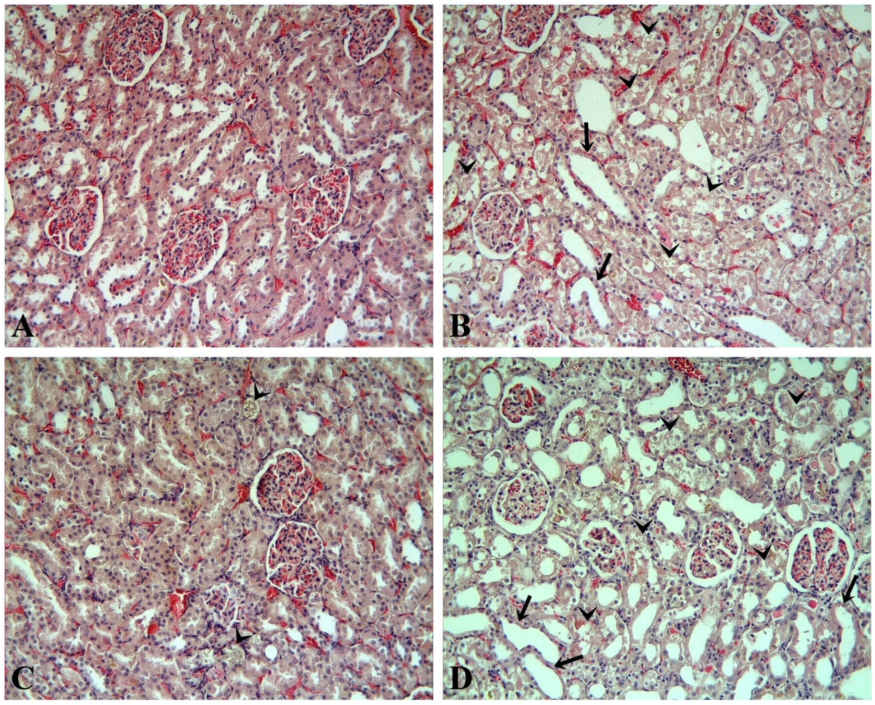
Figure 1. Rat and kidney weights evaluated in the study.

Figure 2. Renal function parameters and electrolytes analyzed in the study.

Figure 3. Representative images of histological evaluation of renal cortical tissue. (A) Control group with normal histological appearance. (B) CIS group showing necrotic changes (arrowheads) and dilatation (arrows) in renal tubules. (C) RAN+CIS group exhibiting a significant decrease in degenerative changes in tubules (arrowheads) compared to the CIS group. Notably, tubular degeneration was more severe in the CIS+RAN group (D) than in the CIS group. H-E staining, magnification x20.

Figure 4. Tissue oxidant and antioxidant parameters measured in the study.





E

Groups	Tubuler degeneration
Control	0.0 (0.0-0.0)
CIS	1.0 (0.0-3.0)[a]
CIS+RAN	3.0 (0.0-3.0)[c]
RAN+CIS	1.0 (0.0-3.0)[b]

Histopathologic scores are expressed as median (min–max).
[a]p<0.0001: significantly increased compared to Control group.
[b]p<0.0001: significantly decreased compared to CIS group.
[c]p<0.0001: significantly increased compared to CIS group.