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Original Article

Harmine mitigates cisplatin-induced renal injury in male mice through antioxidant, anti-inflammatory, and anti-apoptosis effects

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Abstract

Background and purpose: Cisplatin is a chemotherapeutic drug used to treat cancer, however, causes kidney toxicity. Harmine is a plant-derived alkaloid with a wide range of therapeutic applications. The effects of harmine on the renal side effects of cisplatin in mice were studied in this study.
Experimental approach: Forty-eight male BALB/c mice were randomly divided into eight groups (n = 6). They were treated with saline, cisplatin (5.5 mg/kg), harmine (5, 10, and 15 mg/kg/day), cisplatin + harmine (5, 10, and 15 mg/kg/day), respectively. All administrations were done daily and intraperitoneally for 4 days. The criteria related to histology, oxidation, anti-oxidation, inflammation, and apoptosis of renal tissue were evaluated.
Findings / Results: There was a significant decrease in total antioxidant capacity of renal tissue, renal corpuscles diameter, and *IL-10* expression level in the cisplatin group than in the control group, while the values of these parameters were significantly similar to the control group in the moderate or high doses of harmine + cisplatin groups. There were significant increases in serum urea and creatinine levels, bowman space, the amounts of malondialdehyde, apoptosis rate, and TNF-α, NF-κB, IL-1β, and caspase-3 gene expressions in kidney tissue of the cisplatin group compared to the control group, while these criteria did not differ in the moderate or high doses of harmine + cisplatin groups.
Conclusion and implications: Harmine protected the kidneys against cisplatin-induced damage. Antioxidant, anti-inflammatory, and anti-apoptotic harmine properties were involved in this healing effect.

Keywords: Antioxidants; Harmine; Oxidative stress; Toxicity.

INTRODUCTION

Cisplatin is a platinum-based chemotherapy drug used to treat a variety of malignancies in humans, including lung, head and neck, urinary bladder, ovarian, breast, testis, and haematopoietic cancer (1). Although cisplatin is an effective first-line treatment, it has adverse side effects on several organs, including the liver and kidneys (2). Furthermore, approximately one-third of the patients have a lower glomerular filtration rate, increased plasma levels of urea and creatinine, and tubular damage within a few days after receiving cisplatin therapy (2,3). Cisplatin accumulates in the kidney at a higher rate than in other organs, primarily proximal tubular cells. Patients suffering from acute kidney injuries have a higher risk of mortality and are more likely to acquire chronic kidney disease (4).

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The processes of cisplatin-induced nephrotoxicity are complex, involving several pathways. Cisplatin causes autophagy, DNA damage, cytoplasmic organelle failure, use particularly in the endoplasmic reticulum, and mitochondrial damage, all of which activate apoptotic pathways and cause cellular harm through oxidative stress and inflammation (2,3). In other investigations, cisplatin therapy increased oxidative stress in the kidneys of animal models (5-7). However, it is still unclear how the various mechanisms interact and eventually contribute to renal damage.

Antioxidant-rich foods and supplements can help minimize oxidative damage by lowering free radicals in the body (8). Harmine is a β-carboline alkaloid widely existing in some plants like *Banisteria caapi* and *Peganum harmala* L. This plant-alkaloid has a variety of pharmacological and therapeutic properties. These include antioxidant, anti-inflammatory, and antiparasitic properties (9,10). Previous investigations in renal research showed that harmine administration might balance lipid peroxidation and boost kidney tissue's antioxidant capability. As a result, levels of intracellular oxidative stress are reduced (11-13). Furthermore, harmine regulates pro-inflammatory cytokines and transcription factors and promotes apoptosis in malignant cells *via* external and internal mechanisms (14). In line with our results, it has been reported that the use of the flavonoid fraction of *Morus alba,* which has antioxidant activity, with cisplatin can protect kidneys from cisplatin-induced nephrotoxicity (15). Other antioxidants, such as selenium and *Cornus mas* fruit have been shown to minimize the adverse effects of cisplatin in breast cancer patients and the blood serum parameters, respectively (16,17).

Because of the significance of harmine in reducing oxidative stress and inflammation, it can be hypothesized that this plant's compound can reduce the oxidative stress generated by cisplatin in the renal tissue. Harmine's antioxidant impact on cisplatin-induced renal tissue damage has not been studied so far. The present study aims to assess the harmine effects on cisplatin-induced kidney damage in animal samples.

MATERIALS AND METHODS

Chemicals

Harmine (7-methoxy-1-methyl-9H-pyrido [3, 4-b] indole ($C_{10}H_{14}N_2$)) powder was purchased from Sigma Corporation (USA; CAS No: 442-51-3). It was dissolved in saline (0.09%) to reach the desired concentration. Cisplatin (cisplatinum or cis-diamminedichloroplatinum(II) (CDDP); $Cl_2H_6N_2Pt^{+2}$) was purchased from Sigma (USA) and solved in saline (0.09%). Xylazine was purchased from Alfasan Co. Netherlands.

Animals, treatment, and sampling

We employed 48 BALB/c male mice weighed 30 ± 2 g in the current investigation. Animals were housed in separate cages at 22 ± 2 °C with 12/12-h light/dark cycles and access to water and food. This research was approved in agreement with the Helsinki declaration with permission from the Kermanshah University of Medical Sciences Ethics Committee (Ethics No. IR.KUMS.REC.1398.906).

Animals were randomly separated into eight groups. 6 each including (A) control group: receipt normal saline; (B) cisplatin receiving group (5.5 mg/kg) (18); (C-E) harmine-treated groups (5, 10, and 15 mg/kg) (12); (F-H) cisplatin + harmine groups received a mixture of 5.5 mg/kg of cisplatin and harmine at 5, 10, and 15 mg/kg.

The mice were treated with harmine or cisplatin as follows. In the harmine groups, harmine was injected intraperitoneally (IP) for four days. In the cisplatin group, cisplatin was administered as a single dose (IP) at 10 AM. In cisplatin + harmine groups, after injecting cisplatin, harmine was administrated at 11 AM on the same day and three days later (IP). On day five of the experiments, the mice were sacrificed with ketamine and xylazine in deep anesthesia.

Kidneys were set aside for biochemical and molecular research. The blood samples were taken from the heart and centrifuged for 15 min at 4000 rpm. Then the separated serum was transferred to fresh micro-tubes and kept at -20 °C.

Evaluation of the blood serum creatinine and urea nitrogen levels

The samples were centrifuged then the blood serum creatinine and serum creatinine and blood urea nitrogen levels (BUN) levels were determined. Creatinine and BUN concentrations were measured in triplicate using a commercially available test kit (Bioassay System, USA).

Renal histological evaluations

For histological assessment, kidney tissues were fixed in buffer formalin 10% at 4 °C. Then embedded in paraffin, sectioned at 5 µm, and then processed for hematoxylin and eosin (H&E) staining. Images were acquired using a conventional camera and a light microscope after staining (Olympus CH3, Japan) (6).

A blind observer randomly collected at least 150 round or nearly rounded renal corpuscles (400× magnification) with a zigzag approach to get the mean diameters of renal corpuscles, which were then statistically analyzed using a software program (AE-3; Motic S.L.U., Spain). The diameter of each renal corpuscle was calculated as the mean length of two parallel drawing lines that connected the distance between opposing basement membranes of the outer cell layer. The mean of these lines was used to calculate the diameter of the renal corpuscle. In the next step, bowman space was calculated by drawing at least three lines from the outside to inner cell layers of the renal corpuscle and the average was determined (19). In the 50 randomly selected areas of each kidney sample, qualitative alterations such as vascular congestion, dilated distal and proximal tubules, intra-tubular proteinaceous casts, tubular cell detachment, and intra-cellular vacuolization were assessed (6).

Estimating renal levels of malondialdehyde

Malondialdehyde (MDA) levels in the right kidneys were evaluated to determine lipid peroxidation levels. Briefly, the samples were homogenized in a homogenization buffer containing 1.15% KCl and centrifuged for 10 min. Then, a reaction mixture containing sodium dodecyl sulfate (SDS), acetic acid (pH 3.5), thiobarbituric acid, and distilled water were added. The mixture was boiled for 1 h at 95 °C and centrifuged at 3000 *g* for 10 min. Finally, using a spectrophotometer at 550 nm light wavelength, the absorbance rate of the supernatant was obtained.

Evaluating renal levels of total antioxidant capacity

Total antioxidant capacity (TAC) was determined using a kit (TAC 96A) from ZellBio GmbH Germany. It availed oxidation colorimetry resuscitation. In 96-well microplates, the reagent and buffer were combined and processed according to the kit's instructions. A spectrophotometer was used to assess the antioxidant capacity of the specimens at 490 nm. The results were compared to standard ascorbic acid results.

Cell death detection

The induction of apoptosis in kidney tissues was assessed using a cell death detection ELISA-kit (Cat. No.11544675001; Roche Diagnostics GmbH, Mannheim Germany).

Evaluation of inflammatory biomarkers

Total RNA was extracted from kidney tissues using RNAiso Plus (Takara Bio Inc, Japan). PrimeScript RT master mix (Takara Bio Inc, Japan) was used to reverse transcribe the total RNA into cDNA according to the manufacturer's instructions. Quantitative real-time polymerase reaction (qRT-PCR) was performed using SYBR Premix Ex Taq II (Takara Bio Inc., Japan) with a Light Cycler 480 system (Roche, Germany). The mRNA expressions levels of tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), interleukin-1β (IL1β), IL-10, and caspase-3 in kidney tissue were measured. Sequences of the primers were represented in Table 1. Relative gene expression was computed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The data were analyzed using SPSS16. One-way analysis of variance (ANOVA) was used to compare the results, followed by the Tukey post hoc test. The data were reported as mean ± SEM, and *P*-values < 0.05 were considered statistically significant.

Table 1. The sequences of the primers used for reverse transcription real-time quantitative polymerase chain reaction.

Genes	Forward sequences	Reverse sequences
TNF-α	5'-CATGAGCACAGAAAGCATGATCCG-3′	5'-AAGCAGGAATGAGAAGAGGCTGAG-3′
NF-κB	5'-ATGTGGAGATCATGAGCAGC-3'	5'-CCTGGTCCTGTGTAGCCATT-3'
IL-1β	5'-CTTCAGGCAGGCAGTATCACTCAT-3′	5'-TCTAATGGGAACGTCACACACCAG-3′
Caspase-3	5′-ACAGCACCTGGTTACTATTC-3′	5′-CAGTTCTTTCGTGAGCAT-3′
IL-10	5'-CCAAGCCTTATCGGAAATGA-3'	5'-AGGGGAGAAATCGATGACAG-3'
β-Actin	5'-GGCCAACCGTGAAAAGATGA-3′	5'-GACCAGAGGCATACAGGGACA-3′

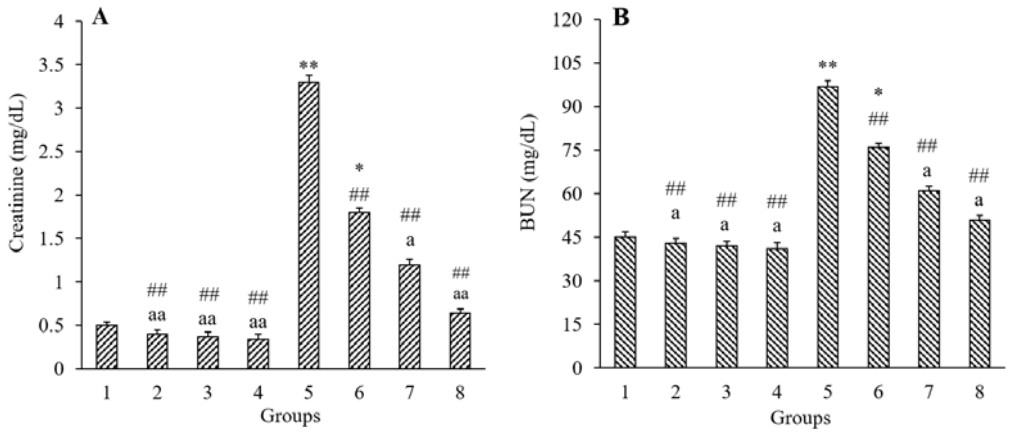

Fig. 1. Effects of harmine (5, 10, and 15 mg/kg) and cisplatin (5.5 mg/kg) treatments on the serum creatinine and BUN levels of kidney in BALB/c mice. Values are presented as mean ± SEM; n = 6. Group 1 (control) received normal saline in a dose of 0.09%; groups 2-4 received harmine at 5, 10, 15 mg/kg, respectively; group 5 treated with cisplatin at 5.5 mg/kg; groups 6-8 received cisplatin at 5.5 mg/kg and harmine at 5, 10, and 15 mg/kg, respectively.*$P < 0.05$ and **$P < 0.01$ indicate significant differences compared to the control group; $^{\#\#}P < 0.01$ versus cisplatin group; $^{a}P < 0.05$ and $^{aa}P < 0.01$ in comparison with cisplatin + harmine (5 mg/kg) group. BUN, Blood urea nitrogen.

RESULTS

Effect of harmine and cisplatin on the serum creatinine and BUN levels

The serum levels of creatinine and BUN were significantly increased in the cisplatin group compared to the control group. The results revealed no significant difference in these two variables between the harmine and control groups. There were no significant differences for harmines intra-group comparison. Serum levels of BUN and creatinine were significantly higher in the cisplatin + harmine 5 mg/kg group than in the control group. Compared to the cisplatin group, all harmine and cisplatin + harmine groups had considerably lower serum creatinine and BUN levels. In comparison with the cisplatin + harmine 5 mg/kg, a significant dose-dependent reduction was observed for these two parameters in the cisplatin + harmine 10 and 15 mg/kg groups (Fig. 1).

Effect of harmine and cisplatin on quantitative histological parameters

The histological examination revealed normal kidney anatomy in both the control and harmine alone-recipient groups. The kidney displayed deformations indicating damage after being treated with cisplatin including, an increase in the Bowman's space, a reduction in the number of glomeruli, inter-tubular hemorrhage, and amplified diameters of the proximal and distal tubules. At all dosages, cisplatin with harmine decreased the kidney damage induced by cisplatin toxicity (Fig. 2A and Table 2). In comparison with the control group, cisplatin decreased the mean diameter of the renal corpuscle and increased the Bowman's space, according to a morphometric study. While, harmine recipient groups at 5, 10, and 15 mg/kg displayed no difference with respect to renal corpuscle diameter and Bowman's space characteristics. When compared to the control group, treatment with cisplatin + harmine 5 mg/kg reduced the

width of the renal corpuscle and increased the Bowman's space. Harmine increased the diameter of the renal corpuscle and decreased Bowman's space in the six harmine-treated groups compared to the cisplatin group. In the cisplatin + harmine group, the diameter of the renal corpuscle increased by incrementing the concentration. In the cisplatin + harmine groups, Bowman's space parameter was considerably lowered when the concentration was increased (Fig. 2B-C).

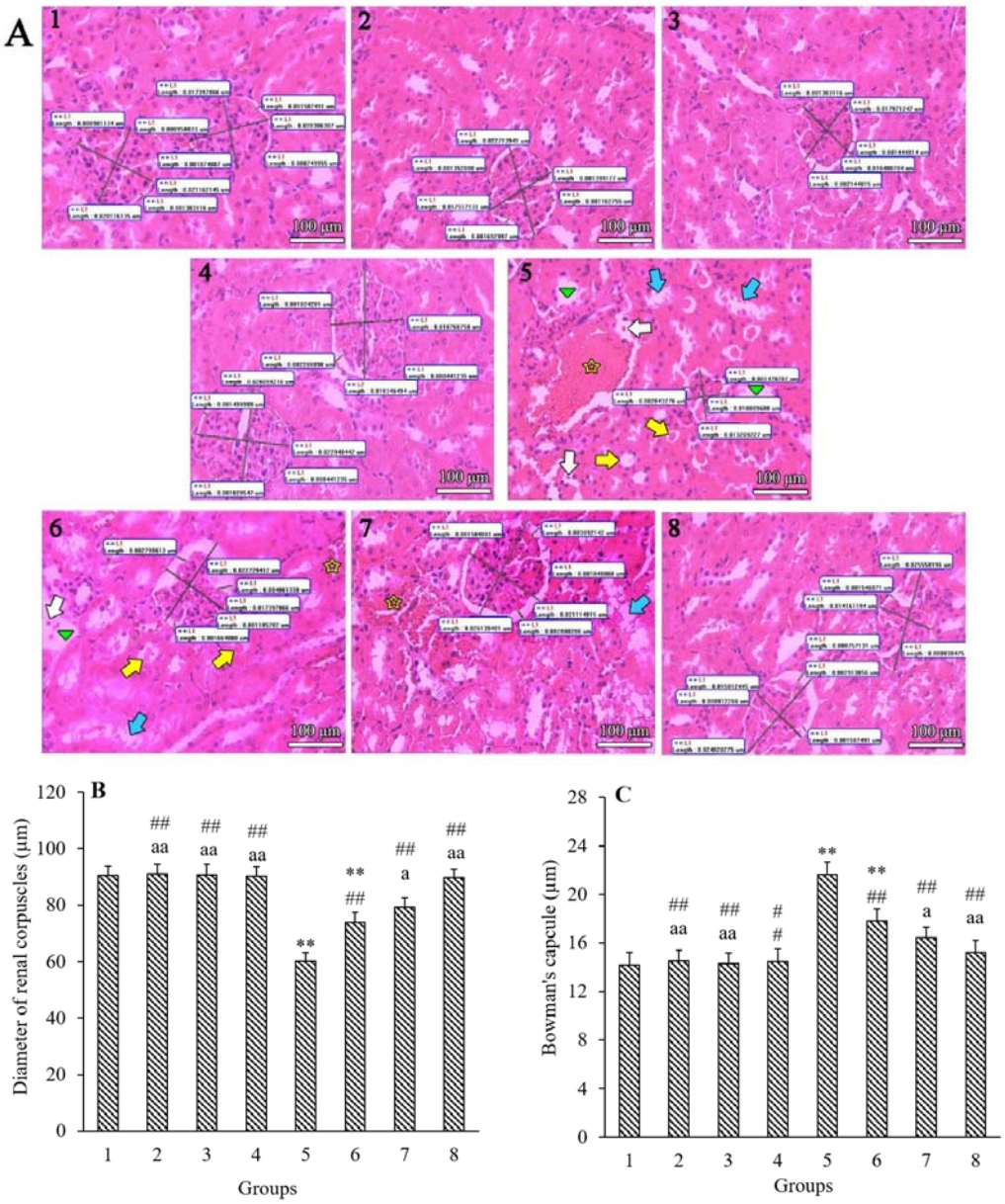

Fig. 2. (A) Histopathological evaluation of kidney tissue in studied groups stained by hematoxylin and eosin (× 400); yellow, blue, and white arrows are showing proteinous cast, intra-cellular vacuolization, and tubular cell detachments, respectively; yellow star is demonstrating vascular congestion; the green triangle is showing intra-tubular dilation of renal tubules. (B and C) Outcomes of harmine and cisplatin treatments on the histological characters in BALB/c mice. Group 1 (control) received normal saline in a dose of 0.09%; groups 2-4 received harmine at 5, 10, 15 mg/kg, respectively; group 5 treated with cisplatin at 5.5 mg/kg; groups 6-8 received cisplatin at 5.5 mg/kg and harmine at 5, 10, and 15 mg/kg, respectively. Values are presented as mean ± SEM; n = 6. $^{**}P < 0.01$ indicates significant differences relative to the control group; $^{\#\#}P < 0.01$ versus cisplatin group; $^{a}P < 0.05$ and $^{aa}P < 0.01$ in comparison with cisplatin + harmine (5 mg/kg) group, respectively.

Table 2. The effect of cisplatin and harmine on changes in the kidney tissue of mice.

Groups	Histopathology indices				
	Intra-cellular vacuolization	Tubular dilatation	Vascular congestion	Intra-tubular proteinaceous casts	Total
1	0	0	I	0	1
2	II	IV	VI	VI	16^{**}
3	0	0	0	0	$0^{aa\neq\neq}$
4	0	0	0	0	$0^{aa\neq\neq}$
5	0	0	0	0	$0^{aa\neq\neq}$
6	I	III	I	0	$5^{*\neq\neq}$
7	0	II	0	0	$2^{a\neq\neq}$
8	0	0	1	0	$1^{aa\neq\neq}$

Group 1 (control) received normal saline in a dose of 0.09%; groups 2-4 received harmine at 5, 10, 15 mg/kg, respectively; group 5 treated with cisplatin at 5.5 mg/kg; groups 6-8 received cisplatin at 5.5 mg/kg and harmine at 5, 10, and 15 mg/kg, respectively. Values are presented as mean ± SEM; n = 6. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences relative to the control group; $^{\#\#}P < 0.01$ versus cisplatin group; $^{a}P < 0.05$ and $^{aa}P < 0.01$ in comparison with cisplatin + harmine (5 mg/kg) group, respectively.

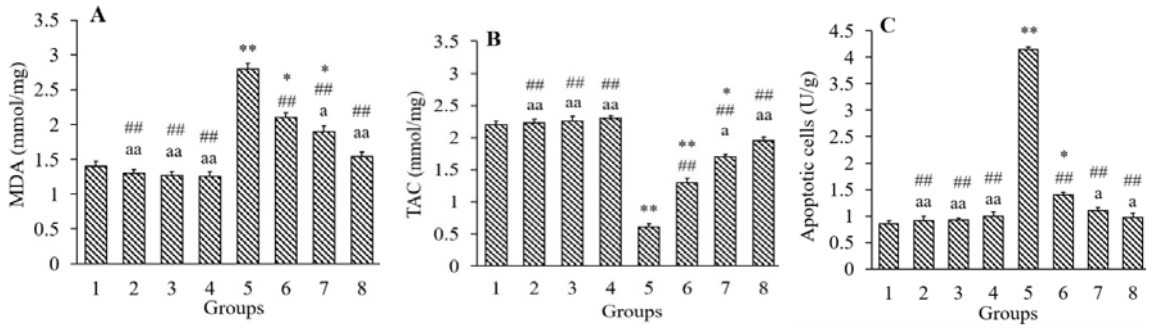

Fig. 3. Outcomes of harmine and cisplatin treatments on the MDA and TAC levels in the kidney tissue and the apoptotic cells in the kidneys of treated-BALB/c mice. Group 1 (control) received normal saline in a dose of 0.09%; groups 2-4 received harmine at 5, 10, 15 mg/kg, respectively; group 5 treated with cisplatin at 5.5 mg/kg; groups 6-8 received cisplatin at 5.5 mg/kg and harmine at 5, 10, and 15 mg/kg, respectively. Values are presented as mean ± SEM; n = 6. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences relative to the control group; $^{\#\#}P < 0.01$ versus cisplatin group; $^{a}P < 0.05$ and $^{aa}P < 0.01$ in comparison with cisplatin + harmine (5 mg/kg) group, respectively. MDA, Malondialdehyde; TAC, total antioxidant capacity.

Effect of harmine and cisplatin on MDA levels in kidney

Measurement of MDA levels of renal tissue in the cisplatin groups revealed a significant increase compared to the control group. Comparing three harmine-treated groups with the control group demonstrated no significant differences in this parameter. Treatment with cisplatin + harmine 5 mg/kg and cisplatin + harmine 10 mg/kg significantly increased this parameter compared to the control group. The MDA levels significantly declined in the harmine-receiving groups compared to the cisplatin group. In the cisplatin + harmine groups, there was a substantial dose-dependent drop in kidney MDA (Fig. 3A).

Effect of harmine and cisplatin on TAC levels in kidney

The renal tissue TAC level showed a significant reduction in the cisplatin group relative to the control group. Compared to the cisplatin group, there were significant increases in the TAC level in kidney tissue in the harmine (5, 10, 15 mg/kg) and cisplatin + harmine (5, 10, 15 mg/kg) groups). This parameter did not indicate any significant differences in the three harmine alone-receiving groups from the control group. Compared to the control group, the cisplatin + harmine 5 mg/kg and cisplatin + harmine 10 mg/kg groups showed a significant decrease in renal TAC levels. In the cisplatin + harmine groups, there was a dose-dependent rise in renal TAC (Fig. 3B).

Effect of harmine and cisplatin on cell death detection

The number of apoptotic cells was significantly more in the cisplatin group than in the control group, as can be seen in Fig. 3C. Compared to the control group, treatment with harmine at 5, 10, and 15 mg/kg had no

significant effect on apoptotic cells. This parameter showed a statistically significant increment between the control and cisplatin + harmine 5 mg/kg groups. Apoptosis cells displayed a considerable reduction in the harmine and cisplatin + harmine 5, 10, and 15 mg/kg groups in comparison with the cisplatin group. There was a significant dose-dependent decrease in apoptotic cells treated with cisplatin + harmine groups.

Effect of harmine and cisplatin on TNFα, NF-κB, IL-1β, IL-10, and caspase-3 mRNA expressions in kidney tissue

TNFα, NF-κB, IL-1β, and caspase-3 mRNA expression levels in mice kidney tissue showed a substantial increase in the cisplatin group compared to the control group. On the other hand, the expression level of IL-10 in the cisplatin group was considerably lower than that in the control group. The biomarkers had no significant difference in the harmine 5, 10, and 15 mg/kg groups compared to the control group. TNFα, NF-κB, IL-1β, and caspase-3 levels were statistically higher in the cisplatin + harmine 5 mg/kg group than in the control group, but IL-10 level was significantly lower. The mRNA expressions of TNFα, NF-κB, IL1β, and caspase-3 in all harmine and cisplatin + harmine groups at all doses were significantly decreased compared to the cisplatin group. The IL-10 expression levels in harmine (5, 10, and 15 mg/kg) and cisplatin + harmine (5, 10, and 15 mg/kg) groups increased compared to the cisplatin group. Regarding intra-group statistical analysis, the expression levels of TNFα, NF-κB, IL1β, and caspase-3 followed the same pattern, in such a way that these parameters were significantly elevated in cisplatin + harmine 5 mg/kg than the cisplatin + harmine 10, 15 mg/kg groups. In the cisplatin + harmine groups, a significant dose-dependent reduction was seen for TNFα, NF-κB, IL-1β, and caspase-3 parameters; in contrast, IL-10 showed a significant dose-dependent increase (Fig. 4A-E).

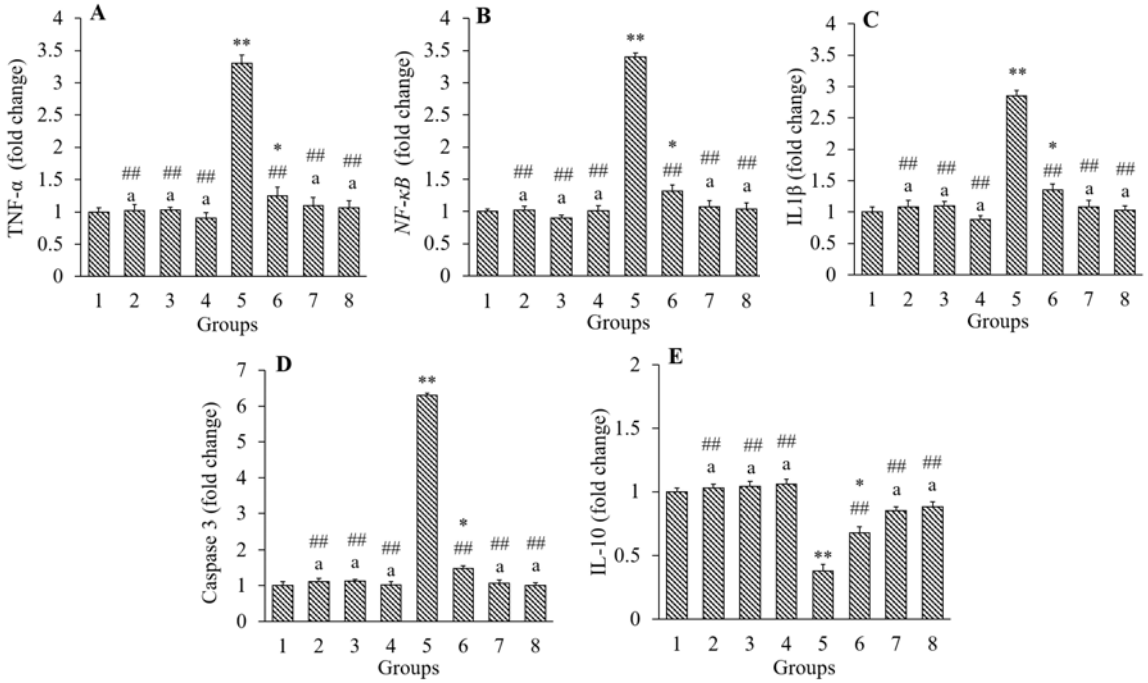

Fig. 4. Effects of harmine (5, 10, and 15 mg/kg) and cisplatin (5.5 mg/kg) treatments on renal levels of (A-E) TNFα, NF-κB, IL-1β, IL-10, and caspase-3 in BALB/c mice. Group 1 (control) received normal saline in a dose of 0.09%; groups 2-4 received harmine at 5, 10, 15 mg/kg, respectively; group 5 treated with cisplatin at 5.5 mg/kg; groups 6-8 received cisplatin at 5.5 mg/kg and harmine at 5, 10, and 15 mg/kg, respectively. Values are presented as mean ± SEM; n = 6. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences relative to the control group; $^{\#\#}P < 0.01$ versus cisplatin group; $^{a}P < 0.05$ in comparison with cisplatin + harmine (5 mg/kg) group, respectively. TNFα, Tumor necrosis factor-α; NF-κB, nuclear factor kappa B; IL, interleukin.

DISCUSSION

Cisplatin is one of the most powerful chemotherapeutic anticancer drugs. Oxidative stress is involved in cisplatin-induced toxicity (2)

Previous research has reported that the β-carboline harmine (one of the main active components of the *Peganum harmala* plant) had antioxidant properties (10). Harmine has been proposed to alleviate cisplatin nephrotoxicity by increasing drug excretion, scavenging free radicals through antioxidant action, and decreasing inflammatory agents. However, no research has been carried out on the antioxidant activity of harmine after cisplatin induction in kidney tissue. In this study, we have investigated the effects of harmine on renal damage induced by cisplatin treatment in male mice. Overall, the current study showed that cisplatin hurts kidney tissue and harmine has a beneficial effect on this side effect of cisplatin, which is more noticeable in moderate and high doses.

In the first phase, we looked at the physiological changes in the kidney when cisplatin and harmine were given alone or together. The progression of serum creatinine and BUN measurements after cisplatin therapy implied that cisplatin hurts renal function. In addition to poor kidney function caused by increased creatinine and BUN levels, glomerular damage caused by decreased renal excretion of this medication is suspected. In line with our results, previous investigations have found that cisplatin therapy raises blood creatinine and BUN levels (6,20). The body's active oxygen metabolism has a dynamic equilibrium in most cases. A considerable amount of active oxygen is produced in the diseased condition. The body's active oxygen metabolism has a dynamic equilibrium in most cases. When the production of antioxidants exceeds the elimination limit, lipid peroxide aggregates, the kidney tubules are damaged, and renal excretory function decreases. Which finally leads to BUN and creatinine retention *in vivo*. These findings reveal a strong link between kidney damage and lipid peroxidation. Increment in the levels of free radicals in the body causes oxidative stress induction, which leads to cell damage and death (21). Our findings showed that harmine generates a considerable decrease in serum levels of BUN and creatinine in a dose-dependent manner. As a result, harmine diminished the harmful effects of cisplatin on renal serum creatinine and BUN levels. This harmine property may be due to its high capacity to scavenge free radicals and thus reduces the rate of oxidation. In agreement with the current data, Jalili *et al*. reported that harmine decreased creatinine and BUN in mercuric chloride-induced mice (12). Another research found harmine to be effective in lowering these two methotrexate-related indicators (13).

The present study demonstrated beside physiology, cisplatin affects histological features of kidney tissue and harmine could relieve it. These data were shown in detail that harmine treatment increased the diameter of renal corpuscles while decreased bowman space in cisplatin recipients. Tubular necrosis prevents urine outflow and increased Bowman's pressure. In this study, it was found that Bowman's space in cisplatin-treated mice increased. Another study came up with similar findings (18). The current study demonstrated cisplatin toxicity on the kidney tissue begins at glomeruli as the point of entry. Then subsequent histological effects were displayed. Urinary space augmentation, glomerular shrinkage, vascular congestion, intracellular vacuolization, tubular dilatation, tubular cell detachments, and intratubular proteinaceous casts are some of the histological alterations.

According to the histological data, the morphology of kidney tissue following harmine treatment was normal, with no evidence of necrosis. However, cisplatin penetration causes edema, lumen enlargement in the proximal and distal tubules, and a change in the morphology of the renal tubule epithelium. Oxidative stress induction in the large region of renal tissues causes all of these damages. Numerous investigations have documented the qualitative effects of cisplatin therapy on renal tissue in laboratory animals (5-7). Harmine treatment also reduced tubular necrosis and glomerular damage in the cisplatin-treated groups. Altogether, harmine effectively restored the cisplatin-induced nephrotoxicity phenotype in

our investigation. The previous study has found that harmine protects kidney tissue against damage caused by nicotine, mercuric chloride, and methotrexate, which is consistent with the current findings (11-13).

The damage to renal tissue after cisplatin administration could be confirmed by a significant rise in renal MDA and a decline in renal TAC levels. These findings may indicate oxidative cisplatin-induced damage in renal tissue. Our outcomes were in line with other studies that demonstrated comparable cisplatin toxicity effects (22). According to the previous findings, oxidative stress is one of the most implicated pathways in the pathogenesis of cisplatin's cytotoxic effects (20,22). In this study, cisplatin-receiving groups with harmine efficiently reduced the MDA and TAC. Harmine has been shown to protect against kidney damage caused by mercuric chloride and methotrexate in previous trials (12,13). Our results support this hypothesis that the cisplatin toxicity mechanism is linked to the depletion of the antioxidant advocacy system.

Apoptosis induction following cisplatin treatment and the cure of this process by harmine can explain another reason for renal-induced toxicity. These data are in accordance with a previous study that showed apoptosis induction by cisplatin (23). During apoptosis, the induction of DNA fragmentation occurs, which results in increased oxidative stress and the following inflammation. According to our findings, harmine suppressed cisplatin-induced apoptosis and necroptosis. These results confirmed the antioxidant activity of harmine to suppress apoptosis in impaired tissues which were accordant with the previous studies (12,24). These expressions indicate why inflammation has occurred in cisplatin-treated mice in the present study. Previous research has shown that these variables exacerbate renal inflammation by activating the immune cell and cytokine cascade (25-27). On the other hand, the present research revealed cisplatin-induced necrosis *via* the TNF-mediated RIPK1/RIPK3/MLKL pathway as reported previously (28). It has been reported that inflammatory cytokine upregulation (such as the TNFα family) was partly decreased in RIPK3-or MLKL-deficient cisplatin-receiving mice (29,30). In the current study, harmine modulated disastrous status caused by cisplatin administration. We found that the beneficial effect of harmine against cisplatin treatment is mediated partially by restoration of histopathological changes, antioxidant effects, inhibitory efficacy on inflammation markers, and anti-apoptosis effects. Also, other researchers have confirmed the anti-inflammatory effects of harmine (10,31).

CONCLUSION

Harmine could reduce cisplatin toxicity in the kidneys. According to the current study, harmine repaired certain kidney-function damages in BALB/c male mice exposed to cisplatin. Furthermore, cisplatin caused histopathological changes alleviated by harmine treatment. The renal therapeutic benefits of harmine on cisplatin-induced kidney damage were mostly due to its antioxidant, anti-apoptotic, and anti-inflammatory activities. The induction of these therapeutic characteristics is more efficient with moderate and high dosages of harmine. As a result, harmine dose-dependently ameliorated the damaging effects of cisplatin on renal tissue.

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Conflict of interest statements

The authors declared no conflict of interest in this study.

Author's contributions

All authors contributed equally to this work. Ali Ghanbari and Cyrus Jalili take responsibility for the integrity of the work as a whole from inception to published article and should be designated as guarantors.

REFERENCES

1. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol. 2021;13:303-328. DOI: 10.2147/JEP.S267383.

2. Pabla N., Dong, Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int. 2008;73(9):994-1007. DOI: 10.1038/sj.ki.5002786.
3. Manohar S, Leung N. Cisplatin nephrotoxicity: a review of the literature. J Nephrol. 2018;31(1):15-25. DOI: 10.1007/s40620-017-0392-z.
4. Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute kidney injury and chronic kidney disease as interconnected syndromes. N Engl J Med. 2014;371(1):58-66. DOI: 10.1056/NEJMra1214243.
5. Santos NA, Catao CS, Martins NM, Curti C, Bianchi MLP, Santos AC. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol. 2007;81(7):495-504. DOI: 10.1007/s00204-006-0173-2.
6. Shokri V, Jalili C, Raissi F, Akhshi N, Ghanbari A. Evaluating the effects of acacetin versus a low dose of cisplatin drug on male reproductive system and kidney in mice: with emphasis on inflammation process. Andrologia. 2020;52(1):e13444,1-9. DOI: 10.1111/and.13444.
7. Tusskorn O, Pansuksan K, Machana K. *Borassus flabellifer* L. crude male flower extracts alleviate cisplatin-induced oxidative stress in rat kidney cells. Asian Pac J Trop Biomed. 2021;11(2):81-88. DOI: 10.4103/2221-1691.303607.
8. Fang C-Y, Lou D-Y, Zhou L-Q, Wang J-C, Yang B, He Q-J, *et al*. Natural products: Potential treatments for cisplatin-induced nephrotoxicity. Acta Pharmacol Sin. 2021;42(12):1951-1969. DOI: 10.1038/s41401-021-00620-9.
9. Babaei Pour A, Moghadar N. Larval effect of extract of harmine and harmalin from Peganum harmala on juvenile of Protostrongylus rufescens. Res Pharm Sci. 2012;7(5):S63.
10. Abbas MW, Hussain M, Qamar M, Ali S, Shafiq Z, Wilairatana P, *et al*. Antioxidant and Anti-Inflammatory Effects of *Peganum harmala* Extracts: An *In Vitro* and *In Vivo* Study. Molecules. 2021;26(19):6084,1-21. DOI: 10.3390/molecules26196084.
11. Salahshoor MR, Roshankhah S, Motavalian V, Jalili C. Effect of harmine on nicotine-induced kidney dysfunction in male mice. Int J Prev Med. 2019;10:97-103. DOI: 10.4103/ijpvm.IJPVM_85_18.
12. Jalili C, Akhshi N, Rashidi I, Ghanbari A. Harmine protects mercuric chloride kidney-induced injury by antioxidant activity in male mice: a biochemical and histological study. Res Pharm Sci. 2020;15(6):541-550. DOI: 10.4103/1735-5362.301339.
13. Jalili C, Darakhshan S, Akhshi N, Abdolmaleki A, Abdi A, Ghanbari A. Harmine has nephroprotective effect against methotrexate-induced injury in mice *via* inhibition of oxidative stress. Res J Pharmacogn. 2021;8(4):9-19. DOI: 10.22127/RJP.2021.272797.1676.
14. Hamsa TP, Kuttan G. Harmine activates intrinsic and extrinsic pathways of apoptosis in B16F-10 melanoma. Chin Med. 2011;6:11-18. DOI: 10.1186/1749-8546-6-11.
15. Nematbakhsh M, Hajhashemi V, Ghannadi A, Talebi A, Nikahd M. Protective effects of the *Morus alba* L. leaf extracts on cisplatin-induced nephrotoxicity in rat. Rese Pharm Sci. 2013;8(2):71-77. PMID: 24019816.
16. Ataei N. Selenium can reduce the side effects of cisplatin as a chemotherapy drug. Res Pharm Sci. 2012;7(5):S94.
17. Mohammadzadeh Vardin A, Abdollahi B, Kosari-Nasab M, Mesgari Abbasi M. Effects of *Cornus mas* fruit hydro-methanolic extract on serum antioxidants, lipid profile, and hematologic parameters following cisplatin-induced changes in rats. Res Pharm Sci. 2017;12(6):510-516. DOI: 10.4103/1735-5362.217431.
18. Raoofi A, Khazaei M, Ghanbari A. Protective effect of hydroalcoholic extract of *Tribulus terrestris* on cisplatin induced renal tissue damage in male mice. Int J Prev Med. 2015;6:11-17. DOI: 10.4103/2008-7802.151817.
19. Chehrei S, Moradi M, Ghiabi HR, Falahi M, Kaviani S, Ghanbari A. Pentoxifylline besides naltrexone recovers morphine-induced inflammation in male reproductive system of rats by regulating Toll-like receptor pathway. Andrologia. 2017;49(9): e12749,1-8. DOI: 10.1111/and.12749.
20. Prabhu VV, Kannan N, Guruvayoorappan C. 1, 2-Diazole prevents cisplatin-induced nephrotoxicity in experimental rats. Pharmacol Rep. 2013;65(4):980-990. DOI: 10.1016/s1734-1140(13)71079-x.
21. Lou X-Y, Cheng J-L, Zhang B. Therapeutic effect and mechanism of breviscapine on cisplatin-induced nephrotoxicity in mice. Asian Pac J Trop Med. 2015;8(10):873-877. DOI: 10.1016/j.apjtm.2015.09.017.
22. Abo El-Magd NF, Ebrahim HA, El-Sherbiny M, Eisa NH. Quinacrine ameliorates cisplatin-induced renal toxicity *via* modulation of sirtuin-1 pathway. Int J Mol Sci. 2021;22(19):10660,1-11. DOI: 10.3390/ijms221910660.
23. Thongnuanjan P, Soodvilai S, Fongsupa S, Chabang N, Vivithanaporn P, Tuchinda P, *et al.* Protective effect of panduratin a on cisplatin-induced apoptosis of human renal proximal tubular cells and acute kidney injury in mice. Biol Pharm Bull. 2021;44(6):830-837. DOI: 10.1248/bpb.b21-00036.
24. Kajbaf F, Oryan S, Ahmadi R, Eidi A. Harmine, a natural β-carboline alkaloid, ameliorates apoptosis by decreasing the expression of caspase-3 in the kidney of diabetic male Wistar rats. Gene Reports. 2020;21:100863,1-10. DOI: 10.1016/j.genrep.2020.100863.
25. Cummings BS, Schnellmann RG. Cisplatin-induced renal cell apoptosis: caspase 3-dependent and-independent pathways. J Pharmacol Exp Ther. 2002;302(1):8-17. DOI: 10.1124/jpet.302.1.8.

26. Arjumand W, Seth A, Sultana S. Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NFκB, TNF-α and caspase-3 expression in Wistar rats. Food Chem Toxicol. 2011;49(9): 2013-2021.
DOI: 10.1016/j.fct.2011.05.012.
27. Li J, Tang Y, Tang PMK, Lv J, Huang X-R, Carlsson-Skwirut C, *et al*. Blocking macrophage migration inhibitory factor protects against cisplatin-induced acute kidney injury in mice. Mol Ther. 2018;26(10):2523-2532.
DOI: 10.1016/j.ymthe.2018.07.014.
28. Xu Y, Ma H-B, Fang Y-L, Zhang Z-R, Shao J, Hong M, *et al*. Cisplatin-induced necroptosis in TNFα dependent and independent pathways. Cell Signal. 2017;31:112-123.
DOI: 10.1016/j.cells ig.2017.01.004.
29. Xu Y, Ma H, Shao J, Wu J, Zhou L, Zhang Z, *et al*. A role for tubular necroptosis in cisplatin-induced AKI. J Am Soc Nephrol. 2015;26(11):2647-2658.
DOI: 10.1681/asn.20140 80741.
30. Wang H, Sun L, Su L, Rizo J, Liu L, Wang L-F. *et al*. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. Mol Cell. 2014;54(1): 133-146.
DOI. 10.1016/j. molcel.2014.03.003.
31. Liu X, Li M, Tan S, Wang C, Fan S, Huang C. Harmine is an inflammatory inhibitor through the suppression of NF-κB signaling. Biochem Biophys Res Commun. 2017;489(3):332-338.
DOI: 10.1016/j.bbrc.2017.05.126.