

Effect of Harmine on Nicotine-Induced Kidney Dysfunction in Male Mice

Abstract

Background: The nicotine content of cigarettes plays a key role in the pathogenesis of kidney disease. Harmine is a harmal-derived alkaloid with antioxidant properties. This study was designed to evaluate the effects of harmine against nicotine-induced damage to the kidneys of mice. **Methods:** In this study, 64 male mice were randomly assigned to eight groups: saline and nicotine-treated groups (2.5 mg/kg), harmine groups (5, 10, and 15 mg/kg), and nicotine (2.5 mg/kg) + harmine-treated groups (5, 10, and 15 mg/kg). Treatments were administered intraperitoneally daily for 28 days. The weights of the mice and their kidneys, kidney index, glomeruli characteristics, thiobarbituric acid reactive species, antioxidant capacity, kidney function indicators, and serum nitrite oxide levels were investigated. **Results:** Nicotine administration significantly improved kidney malondialdehyde (MDA) level, blood urea nitrogen (BUN), creatinine, and nitrite oxide levels and decreased glomeruli number and tissue ferric reducing/antioxidant power (FRAP) level compared to the saline group ($P < 0.05$). The harmine and harmine + nicotine treatments at all doses significantly reduced BUN, kidney MDA level, creatinine, glomerular diameter, and nitrite oxide levels and increased the glomeruli number and tissue FRAP level compared to the nicotine group ($P < 0.05$). **Conclusions:** It seems that harmine administration improved kidney injury induced by nicotine in mice.

Keywords: Harmine, kidney, nicotine

Introduction

Consumption of nicotine in the form of cigarettes or other nicotine-based products, such as the hookah or pipe, is prevalent, and nicotine addiction is the main reason for tobacco consumption.^[1] As a member of Solanaceae family, nicotine is an alkaloid formed by pyridine and pyrrolidine loops found in the tobacco plant.^[2] Oxidative stress and an increase in lipid oxidase production occur following nicotine injection and may lead to irreversible damage of the cellular membrane.^[3] Increased levels of oxidative stress result in an imbalance in free radical generation and antioxidant defense. This imbalance, in turn, results in the oxidation of biomolecules and changes their structure and function.^[4] Gopaul *et al.* showed that the lipid oxidase level increases in cigarette smokers.^[5] The increased level of reactive oxygen species (ROS) causes oxidative stress and induces DNA breakage. In turn, as particular proteins become deactivated, biological membranes degenerate.^[6] Kidney is the main

metabolizing organ that discharges toxins in the urine. The primary or secondary discharge of some toxins and drugs may result in chronic kidney disorders.^[7] Smoking cigarettes has a negative effect on the kidneys. Cigarettes can increase albumin discharge and proteinuria and cause kidney malfunction.^[8] Antioxidant supplements and antioxidant-rich foods can reduce free radicals in the human body and decrease oxidative damage.^[9] *Peganum harmala* is a member of the Zygophyllaceae family. It grows in many countries in North Africa and the Middle East. It contains alkaloids which are generally found in its seeds and root.^[10] Harmine and harmaline are the most important alkaloids available in *P. harmala* that have positive effects.^[11] The essence of this plant can be used to treat bradycardia, decrease blood pressure, control angiogenesis, and has antiallergic, antispasm, and antiadrenergic effects.^[12] Traditional medicine has listed its positive effects as being a soporific emmenagogue, appetite inducer, and antiparasitic.^[13] Harmine is an active component of *P. harmala* and is a herbal alkaloid of the beta-carboline family.

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It is extracted from *P. harmala* and is known to have pharmacologic effects, especially as an antioxidant.^[14] It is a strong controller of tyrosine phosphorylation-regulated kinase and shows cytotoxic activity against tumor cells in the human body.^[15] Harmine can induce apoptosis and regulate transcription factors and preinflammatory cytokines.^[16] In addition, it can suppress tumor necrosis factor- α activity as well as nitrite oxide production.^[17] Nitrite oxide is a signaling molecule that plays a significant role in biological systems. Nicotine absorption in the body appears to increase the serum levels of nitrite oxide and oxidative stress.^[18] Nicotine has a toxic effect and harmine has advantageous properties.^[19] No study has evaluated the antioxidant effect of harmine on kidney damage induced by nicotine. The current study was designed to evaluate the effect of harmine on kidney disorders and damage induced by nicotine in animal samples.

Methods

Chemicals

In this experimental study, harmine (7-methoxy-1-methyl-9h-pyrido (C₁₀H₁₄N₂)) powder was purchased (from Sigma, USA). The powder was diluted with saline (0.9%) to obtain the desired doses. Nicotine (CAS Name: 3-[(2S)-1-methyl-2-pyrrolidinyl]pyridine) was purchased from Sigma (USA) and dissolved in saline (0.9%) for administration.^[1,14]

Experimental animals

A total of 64 male BALB/c mice weighting 27–30 g were purchased from Razi Institute (Iran). All the mice were housed in plastic cages in a room at 23 \pm 2°C, under controlled environmental conditions, a 12/12 h light/dark cycle, with free access to water and food. All investigations conformed to the ethical and humane principles of research and were approved by the Ethics Committee of Kermanshah University of Medical Sciences (Ethics Certificate No. 1395.38).^[6]

Protocol and treatments

The animals were randomly separated into eight groups ($n = 8$). Group 1 was for saline (normal saline; 1 ml DW/daily); group 2 was for nicotine (2.5 mg/kg);^[11] groups 3 to 5 were treated with harmine in dosages of 5, 10, and 15 mg/kg, respectively;^[20] groups 6 to 8 were treated with harmine in dosages of 5, 10, and 15 mg/kg, and nicotine (2.5 mg/kg). Nicotine was administered intraperitoneally (IP) daily for 4 weeks. Harmine and harmine + nicotine were administered in the same manner.

Weight of mice, kidney, kidney index, and collection of blood serum

The body weight was measured at the onset and end of the study. Animals of each group were placed one after another in a plastic container in a packet of cotton covered with

ether for 24 h. They were anesthetized due to inhalation of ether fume. Venipuncture from the animals' hearts (right ventricle) was done. The blood sample was incubated for 15 min at 37°C to clot. Then the clotted blood was centrifuged for 15 min at 3000 rpm until the serum was separated. The separated serum was stored at a temperature of -70°C for measurement of the nitrite oxide, creatinine, and blood urea nitrogen (BUN). The mice were then sacrificed. The kidneys were removed and weighed on a microbalance sensitive up to 0.001 mg (Precisa 125A; Switzerland) and the average weights of the kidneys were calculated and recorded. The renal index was derived from the division of the total weight of the two left and right kidneys into the total weight of the mice.^[9]

Histological and morphometric analysis

The kidney was divided into two equal parts from the middle by a cross-section after the samples were fixed by 10% formalin solution and washed. The central part was immersed in 70% alcohol, and the process of tissue preparation was based on the conventional histology method. Serial sections were prepared (5 μ m thick) using a microtome (EC350-2). Hematoxylin and eosin staining methods were used to stain the nucleus purple and the cytoplasm pink. The diameter and number of glomeruli were examined under the Olympus BX-51T-32E01 microscope linked to a DP12 camera with 3.34-million pixel resolution and Olysia Bio software (Olympus Optical, Japan).^[6]

Biochemical marker assays

The blood taken from the heart was incubated at 37°C for 15 min and centrifuged at 3000 rpm for 15 min to acquire the serum. The serum samples were kept in a -20°C freezer. Plasma samples were assayed for concentrations of creatinine and BUN using an autoanalyzer (RA 1000; Technicon Instruments, USA).^[21]

Griess technique

Griess technique uses zinc sulfate powder to eliminate the serum protein of the samples. Accordingly, zinc sulfate powder (6 mg) was mixed with serum samples (400 μ l), and vortexed for 1 min. After centrifugation of the samples (10 min at 12,000 rpm), the supernatant was used to measure the nitrite oxide. Briefly, 50 μ l of sample was added to 100 μ l of Griess reagent (Sigma, USA) and the reaction mixture was incubated for about 30 min at room temperature. The sample optical density was measured by enzyme-linked immunosorbent assay reader (Hyperion, USA) at a wavelength of 450 nm according to the manufacturer protocol.^[11]

Ferric reducing/antioxidant power (FRAP) and malondialdehyde (MDA) assays

To evaluate oxidative stress by performing colorimetric analysis, the thiobarbituric acid reactive species were

measured by means of MDA as the last product of lipid peroxidation in renal tissue. The renal antioxidant capacity was measured by FRAP analysis. The FRAP substance contained 1.5 ml chloride ferric (Sigma, USA) and 30 ml of acetate buffer (Sigma, USA). Serial concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma, USA) were used as an external standard.^[21]

Statistical analysis

Statistical comparisons among groups were investigated by one-way analysis of variance, followed by the least-square difference *post-hoc* test. A *P* value < 0.05 was considered significant. The SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Weight of kidneys, animals, and kidney index

Harmine was shown to improve the kidney index, animal weight, and kidney weight in animals treated at all doses compared with the nicotine group (*P* < 0.05). The mean kidney index and mean animal and kidney weights showed no significance in animals treated with harmine at all doses in comparison with the saline group (*P* > 0.05). The mean kidney index and mean animal and kidney weights decrease significantly in animals treated with harmine + nicotine at all doses in comparison with the saline group (*P* < 0.05). The mean kidney index and mean animal and kidney weights increased significantly in animals treated with harmine and harmine + nicotine at all doses in comparison with the nicotine group (*P* < 0.05). Moreover, the effective dose of nicotine significantly decreased the mean kidney

index and mean animal and kidney weight compared to the saline group (*P* < 0.05) [Figure 1].

Histological and morphometric analysis

Histological analysis showed normal kidney structure in the saline and harmine treatment groups. After treatment with nicotine, the kidney showed evident changes and injury. These anomalies included an increase in the Bowman's capsule, decrease in the number of glomeruli, intertubular bleeding, and enlarged diameters of the distal and proximal tubules. Treatment with nicotine + harmine at all doses reduced the kidney damage caused by nicotine toxicity [Figure 2]. Morphometric analysis revealed that nicotine significantly increased the mean diameter of the glomerulus tubule and decreased the glomeruli number compared with the saline group (*P* < 0.05). Treatment with harmine significantly increased the diameter of the glomeruli (*P* < 0.05) but no significance in the number of glomeruli in all treatment groups compared with the saline group (*P* > 0.05). Treatment with harmine + nicotine significantly decreased the number of glomeruli (*P* < 0.05) but not significant in the diameter of the glomeruli in all treatment groups compared with the saline group (*P* > 0.05). Treatment with harmine and nicotine + harmine significantly decreased the diameter of the glomeruli but increased the number of glomeruli in all treatment groups compared with the nicotine group (*P* < 0.05) [Figure 3].

Biochemical marker

Nicotine (2.5 ml/kg) significantly increased the mean plasma BUN and creatinine concentration compared to

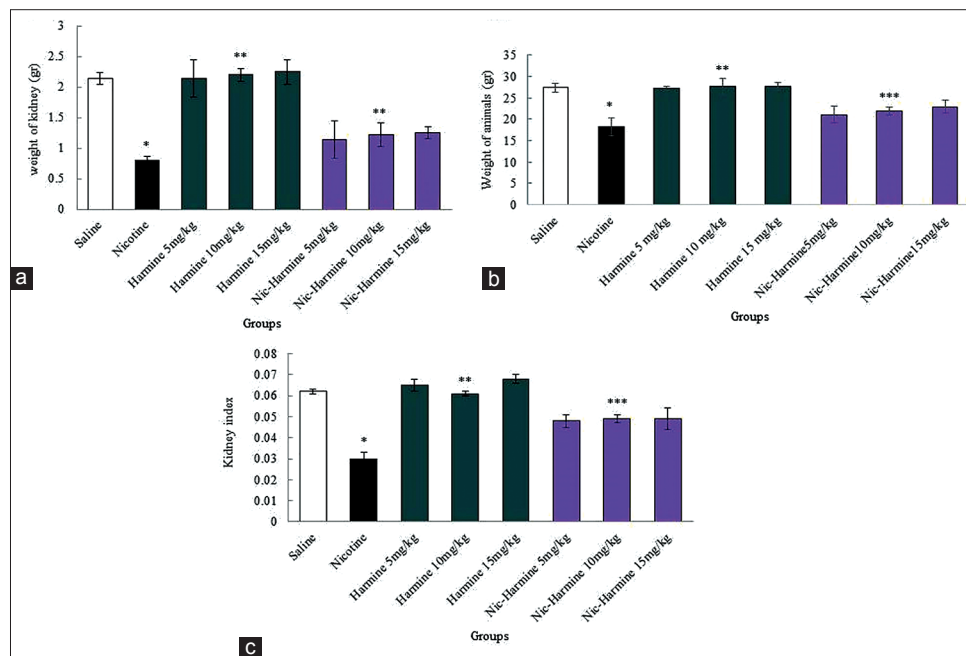


Figure 1: Effect of nicotine, harmine, and harmine + nicotine on weight of (a) kidney; (b) animals; (c) kidney index. *Significant decrease in weight and kidney index of nicotine group compared to saline group (*P* < 0.05). **Significant increase in all harmine groups compared to nicotine group (*P* < 0.05). ***Significant increase (compared to nicotine group) and decrease (compared to saline group) in all harmine + nicotine groups (*P* < 0.05)

the saline group ($P < 0.05$). The mean plasma BUN and creatinine concentration showed no significance in all harmine groups compared with the saline group ($P > 0.05$).

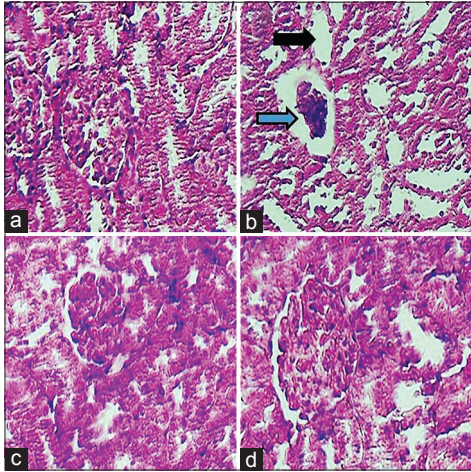


Figure 2: Histological changes in kidneys (hematoxylin-eosin, x 400): (a) Normal kidney, saline group; (b) nicotine group, increased Bowman's capsule space and glomerular shrinkage (blue arrow) and increased distal and proximal tubule diameter (black arrow); (c) normal kidney, harmine 15 mg/kg group showing glomeruli with Bowman's capsule and distal and proximal tubules; (d) normal kidney structure in nicotine + harmine 15 mg/kg group

The mean plasma BUN and creatinine concentration increased significantly in all harmine + nicotine groups compared with the saline group ($P < 0.05$). The mean plasma BUN and creatinine concentration decreased significantly in all harmine and harmine + nicotine groups compared with the nicotine group ($P < 0.05$) [Figure 4].

Nitrite oxide

The mean nitrite oxide in the blood serum increased significantly in the nicotine (2.5 ml/kg) group compared with the saline group ($P < 0.05$). The mean nitrite oxide in the blood serum is not significant in all harmine and harmine + nicotine groups compared with the saline group ($P > 0.05$). The mean nitrite oxide in the blood serum decreased significantly in all harmine and harmine + nicotine groups compared with the nicotine group ($P < 0.05$) [Figure 5].

Oxidative stress

The results of the oxidative stress testing in the groups showed that the kidney MDA level significantly increased in the nicotine group compared to the saline group ($P < 0.05$). Harmine decreased significantly the kidney MDA level in all treatment groups compared to

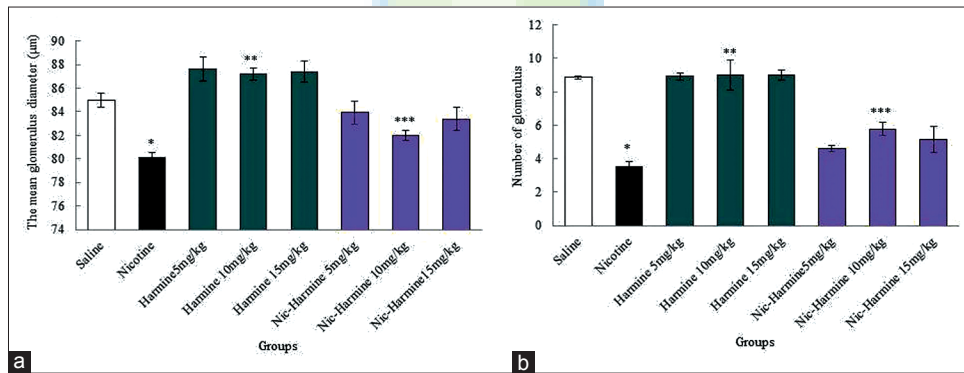


Figure 3: Correlation between treatment groups for (a) glomerular diameter; (b) glomeruli number. *Significant increase in nicotine group compared to saline group ($P < 0.05$). **Significant increase in all harmine groups compared to nicotine and saline (in glomerular diameter) groups ($P < 0.05$). ***Significant increase (compared to nicotine group) and decrease (compared to saline group) in all harmine + nicotine groups ($P < 0.05$)

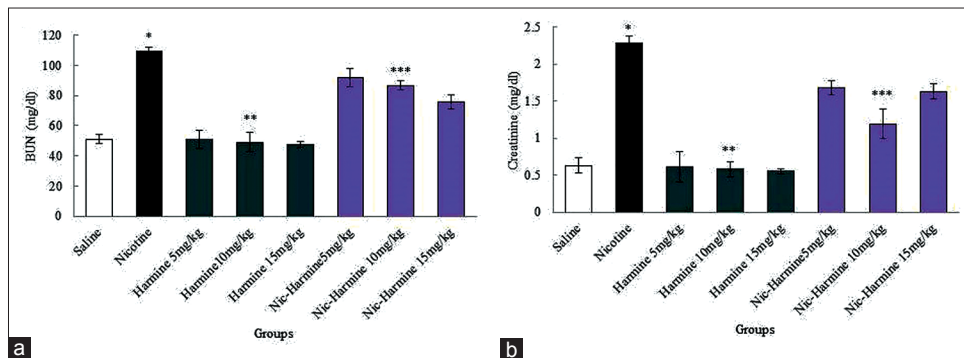


Figure 4: Effect of nicotine, harmine, and harmine + nicotine on the mean kidney biochemical factors: (a) BUN (b) creatinine. *Significant increase in biochemical factor in nicotine groups compared to saline groups ($P < 0.05$). **Significant decrease in biochemical factors of all of harmine groups compared to nicotine groups ($P < 0.05$). ***Significant decrease (compared to nicotine group) and increase (compared to saline group) in biochemical factors of all harmine + nicotine groups ($P < 0.05$)

the nicotine group ($P < 0.05$). The kidney MDA level decreased significantly in all harmine + nicotine groups compared to the nicotine group ($P < 0.05$). Similarly, nicotine significantly decreased the renal tissue FRAP level of the nicotine group in comparison with that of the saline group ($P < 0.05$). Administration of harmine significantly increased the FRAP level in the kidney tissue in all harmine and harmine + nicotine groups compared to the nicotine group ($P < 0.05$). Treatment with harmine in all groups showed no significant value in the renal tissue FRAP level and kidney MDA level compared to the saline group ($P > 0.05$). Treatment with harmine + nicotine significantly increased the kidney MDA level ($P < 0.05$) and not significant in the renal tissue FRAPs level in all treatment groups compared to the saline group ($P > 0.05$) [Figure 6].

Discussion

Chronic kidney disease is a general health problem. The nicotine content of cigarettes has important biological effects and plays a key role in the pathogenesis of kidney disease.^[22] Harmine is an herbal alkaloid from the

beta-carboline family which is extracted from *P. harmala* and different pharmacological effects.^[14] The current study evaluated the protective effects of harmine on disorders induced by nicotine in mice. The results showed a significant decrease in the kidney index, weight of the kidney, and the whole body of nicotine-receiving mice compared with the saline group. In the harmine + nicotine groups, there was a significant increase in the kidney index, weight of the kidney, and whole body of the mice compared with the nicotine group. It appears that nicotine releases dopamine, serotonin, and γ -amino butyric acid, suppresses the appetite, and increases metabolism.^[23] Arany *et al.* showed that prescribing nicotine to mice with a high-fat diet significantly decreased their body weight and body mass index. This agrees with the results of the current study.^[24] It appears that nicotine causes metabolism-induced generation of free radicals, lipid peroxidation, reacts with DNA and membrane proteins, and causes cell damage, which are the main causes of weight and kidney index loss.^[1] The results of Hamden *et al.* agree with the findings of this study that *P. harmala* recovered weight loss in rats treated with thiourea.^[25] It appears that harmine improved food absorption by bonding to receptors such as mono-amino-oxidase (MAO-A), serotonin 2A, and kinase-dependent syncline and increased their weight and kidney index.^[26] In the current study, nicotine alone increased the diameter of the glomeruli and decreased the number of glomeruli. In groups treated with nicotine + harmine, the diameter of the glomeruli decreased and the number of glomeruli increased significantly compared to groups receiving only nicotine. It appears that kidney glomeruli are very sensitive to oxidative stress.^[6] Nicotine is a strong carcinogen that is oxidized into cotinine metabolites largely in the liver, kidney, and lung. Cotinine can play a critical role in the pathogenesis of tissue injury.^[27] It appears that nicotine can induce cytochrome P450, produce free radicals, and generate oxidative stress in tissues.^[3] Fat peroxidation and the production of additional free radicals can damage proteins and DNA, and can induce apoptosis in kidney tissue cells.^[9]

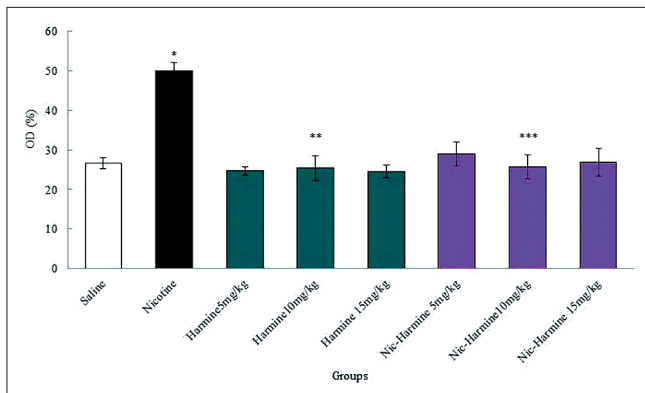


Figure 5: Effects of harmine, nicotine, and harmine + nicotine on mean nitrite oxide levels. *Significant increase in nitrite oxide in nicotine group compared to saline group ($P < 0.05$). **Significant decrease in all harmine groups compared to nicotine group ($P < 0.05$). *Significant decrease in all harmine + nicotine groups compared to nicotine group ($P < 0.05$)**

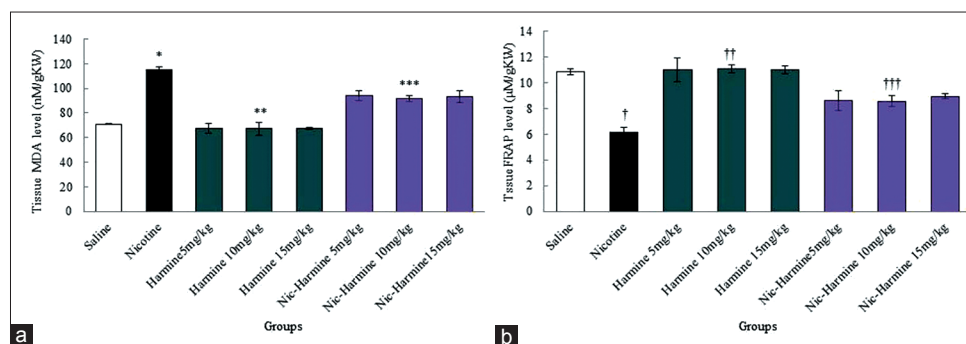


Figure 6: Comparison of nicotine, saline, and harmine + nicotine groups of (a) kidney MDA level; (b) tissue FRAP level. *Significant increase in nicotine group compared to saline group ($P < 0.05$). **Significant decrease in all harmine groups compared to nicotine group ($P < 0.05$). *Significant decrease (compared to nicotine group) and increase (compared to saline group) in all harmine + nicotine groups ($P < 0.05$). †Significant decrease in nicotine group compared to saline group ($P < 0.05$). ††Significant increase in all harmine groups compared to nicotine group ($P < 0.05$). †††Significant increase (compared to nicotine group) and decrease (compared to saline group) in all harmine + nicotine groups ($P < 0.05$)**

The reduced mean diameter and number of glomeruli are found in kidney function disorders. ROS and proxy nitrite can intensify vascular and tubule damage.^[6] Najafi *et al.* reported that an increased concentration of cytosol calcium due to oxidative stress-induced mitochondrial damage in the cellular skeleton interfered with the metabolism of mitochondrial energy by activating proteases, endonucleases, and phospholipases, which resulted in necrosis of tubular epithelial cells.^[28] This agrees with the results of the current study. Because of its antioxidant effect, it appears that harmine largely neutralized the effect of nicotine for the number and diameter of glomeruli in this study^[20] where the sole prescription of nicotine increased BUN and creatinine in the blood serum of the study groups. In the nicotine + harmine-receiving group, there was a significant decrease in the creatinine and BUN levels compared to the nicotine group. The increased BUN and creatinine may serve as the signal of glomerulus damage induced by decreased discharge of substances from the kidney.^[6] It appears that induced oxidative stress and increased production of free radicals resulted in glomerulus necrosis and affected kidney filtration capacity.^[29] Jalili *et al.* showed that nicotine increases BUN and creatinine in their study animals, which agrees with the results of the current study.^[9] The decreased oxidation speed, thanks to the higher capacity of this alkaloid to eliminate free radicals, appears that harmine can reduce the effect of nicotine to reduce glomerulus damage and increase the serum level of BUN and creatinine.^[30] Measurements of nitrite oxide level of the blood serum indicated a significant increase in the serum level of nitrite oxide in the nicotine group compared to saline group. In this study, the prescription of harmine + nicotine significantly decreased the nitrite oxide level in the study groups. It appears that nicotine stimulates the generation of nitrite oxide by stimulating the release of noradrenaline in the paraventricular and amygdala nucleus and by direct influence on the solitary nuclei.^[31] Nitrite oxide can increase the excessive entry of calcium to cellular cytosol and induce a toxic effect on cells.^[32] The excessive production of nitrite oxide and the increased iNOS and nNOS expression may induce nephrotoxicity, nephritic diseases, and nephrotoxic disease.^[9] It appears that NOS isoform is expressed in the kidney and its increased expression increases the thickness of the distal tube, proximal tubule, and urine collecting tracts.^[33] Antioxidants can reduce the production of nitrite oxide through degeneration and damage to the nitrite oxide system.^[34] The results of El Madani *et al.* show that harmine prescription significantly decreased the serum level of nitrite oxide in the mice treated by rotenone.^[35] The results of the current study reveal that harmine as an antioxidant can lead to the relative mitigation of nicotine-induced damage kidney tissue. In addition, it appears that harmine mitigates the induction of inflammation and kidney damage in the study animals by reducing the serum level of nitrite oxide and increasing the total antioxidant capacity of the

body by controlling nuclear factor-K β expression. The consequences of the current study similarly displayed that treatment by harmine is able to moderate lipid peroxidation and increase antioxidant capacity of renal tissue, and consequently reduces oxidative stress. Therefore, it seems that antioxidant properties of harmine via preventing the creation of ROS could increase FRAPs and decrease MDA levels in the studied groups.

This study revealed the positive effects of harmine in reducing the levels of blood urea and the effects of creatinine that serves as kidney damage indices. For the complete information about the potential effects demand wider supplementary studies that fully identify the molecular and cellular mechanism(s) affecting the pharmacological functions of this substance when treating and controlling organ damage in smokers or those with other diseases with increased oxidative stress etiology.

Conclusions

The results of this study indicate that harmine may recover some kidney function in mice treated with nicotine. It could be valuable for protection of the kidney in individuals who have been exposed to nicotine. The antioxidant properties of harmine perhaps is the main cause of therapeutic effect on some renal parameters, but supplementary studies are essential to describe its molecular mechanism.

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Conflicts of interest

There are no conflicts of interest.

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