



Harmine recover kidney damage induced by morphine in male rats

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ABSTRACT

Harmine is a harmal-derived alkaloid with antioxidant properties. The morphine produces free radicals and plays a key role in the pathogenesis of kidney disease. This study was designed to evaluate the effects of harmine against morphine-induced damage to the kidneys of rats. In this study, 64 male rats were randomly assigned to 8 groups: saline and morphine treated groups; harmine groups (5, 10, 15 mg/kg) and morphine + harmine treated groups (5, 10, 15 mg/kg). Treatments were administered intraperitoneally daily for 20 days. The weights of the animals and their kidneys, kidney index, glomeruli characteristics, thiobarbituric acid reactive species, antioxidant capacity, kidney function indicators and serum nitrite oxide levels were investigated. Morphine administration significantly improved kidney MDA level, blood urea nitrogen (BUN), creatinine and nitrite oxide levels and decreased glomeruli number and tissue FRAP level compared to the saline group ($P < 0.05$). The harmine and harmine + morphine 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 morphine group ($p < 0.05$). It seems that harmine administration improved kidney injury induced by morphine in rats.

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Keywords

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INTRODUCTION

Opioids produce free radicals and cause apoptosis in some cell. Morphine is an opioid analgesic drug, and the main psychoactive chemical in opium [1]. Morphine is addictive cause physiological dependence [2]. Oxidative stress and an increase in lipid oxidase production occurs following morphine 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]. Long-term opioid use is associated with undesirable consequences including renal function [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 de-

generate [6]. The 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]. Morphine 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 anti-allergic, anti-spasm and anti-

adrenergic 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 an herbal alkaloid of the beta-carboline family. 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 (DYRK) and shows cytotoxic activity against tumor cells in the human body [15]. Harmine can induce apoptosis and regulate transcription factors and pre-inflammatory cytokines [16]. In addition, it can suppress TNF- α activity as well as nitrite oxide production [17]. Nitrite oxide is a signaling molecule that plays a significant role in biological systems. Morphine absorption in the body appears to increase serum levels of nitrite oxide and oxidative stress [18]. Morphine has a toxic effect and harmine has advantageous properties [19]. No study has evaluated the antioxidant effect of harmine on kidney damage induced by morphine. The current study was designed to evaluate the effect of harmine on kidney disorders and damage induced by morphine in animal samples.

MATERIALS AND METHODS

Experimental Animals

A total of 64 male rats weighting 250–270 g were purchased from Razi Institute (Iran). All the rats were housed in plastic cages in a room at $23\pm 2^{\circ}\text{C}$, 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

A total of 64 male rats were randomly divided into 8 groups and 8 rats were placed in each group. The first group was saline (control) group, which received normal saline through intraperitoneal injection equivalent to the amount of experimental groups. In the second Morphine group, Morphine administered via interaperitoneally injecting (20 mg/kg once daily in the first five days and double per day in the following five days. On the eleventh to twentieth day, a dose of up to 30 mg /kg doubles each day) [5]. The third to fifth groups included the harmine groups, in which each animal respectively received 5, 10, and 15 mg/kg of harmine once daily, intraperitoneally, on days 1–20 [20]. The sixth to eighth groups include Morphine + harmine groups, in which each animal on days 1–20, received 5, 10, and 15 mg/kg of harmine once daily plus Morphine intraperitoneally.

Weight of rats, kidney 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 24 hours. 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

minutes at 37°C to clot. Then the clotted blood was centrifuged for 15 minutes at 3000 rpm until the serum was separated. The separated serum was stored at the temperature of -70°C for measurement of the nitrite oxide, creatinine and blood urea nitrogen (BUN). The rats 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 [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 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 an 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 μl), and vortexed for 1 min. After centrifuged the samples (10 min at 12,000 rpm), the supernatant was used to measure the nitrite oxide. Briefly, 50 μl of sample was added to 100 μl of griess reagent (Sigma; USA) and the reaction mixture was incubated for about 30 min at room temperature. The sample optical density (OD) was measured by ELISA reader (Hyperion; USA) at a wavelength of 450 nm according to manufacturer protocol [1].

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

To evaluation of oxidative stress, through 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 analyze. The FRAP substance was contained of 1.5ml 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 via one-way analysis of variance (ANOVA), followed by the LSD post hoc test. A value of $P < 0.05$ was considered significant. The SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA). was applied for statistical analysis.

RESULTS

Weight of kidneys and animals

Harmine was shown to improve the animal weight and kidney weight in animals treated at all doses compared to the morphine group ($P < 0.05$). The mean animal and kidney

weights no significant in animals treated with harmine at all doses in comparison with the saline group ($P > 0.05$). The mean animal and kidney weights decrease significantly in animals treated with harmine + morphine at all doses in comparison with the saline group ($P < 0.05$). The mean animal and kidney weights increased significantly in animals treated with harmine and harmine + morphine at all doses in comparison with the morphine group ($P < 0.05$). Moreover, the effective dose of morphine significantly decreased the mean animal and kidney weight compared to the saline group ($P < 0.05$) (Fig. 1).

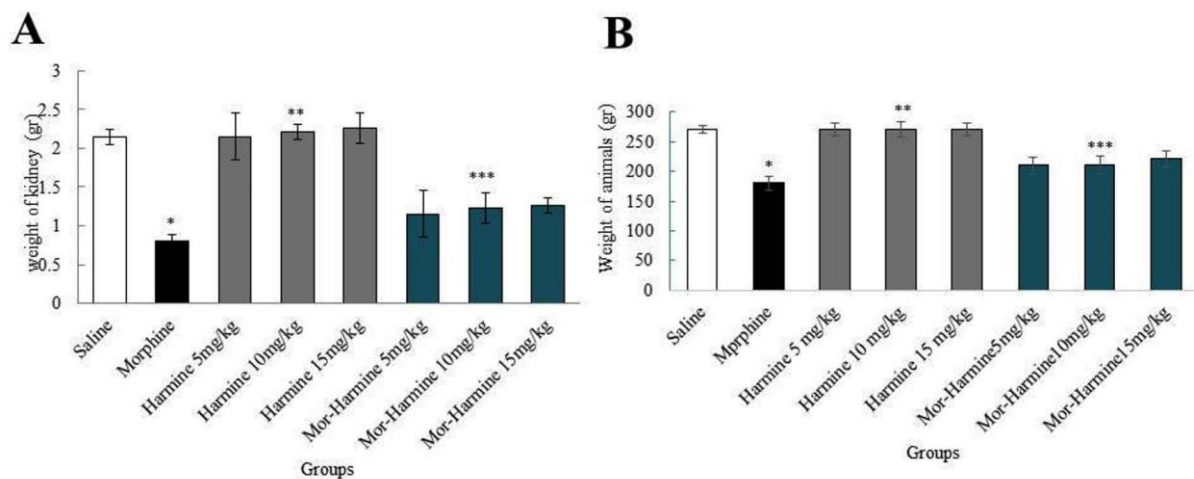


Figure 1. Effect of morphine, harmine and harmine + morphine on weight of: (A) kidney; (B) animals; *Significant decrease in morphine group compared to saline group ($P < 0.05$). **Significant increase compared to morphine group ($P < 0.05$). ***Significant increase (compared to morphine group) and decrease (compared to saline group) for all harmine + morphine groups ($P < 0.05$).

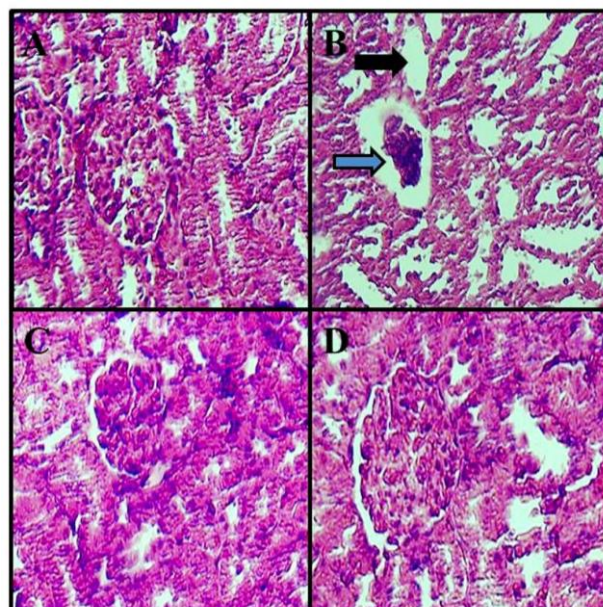


Figure 2. Histological changes in kidneys (hematoxylin-eosin, $\times 400$): (A) Normal kidney, saline group; (B) morphine group, increased Bowman's capsule space and glomerular shrinkage (blue arrow) and increased distal and proximal tubule diameter (black arrow); (C) Normal kidney, harmine 15 mg/kg group showing glomeruli with Bowman's capsule and distal and proximal tubules; (D) normal kidney structure in morphine + harmine 15 mg/kg group.

Histological and morphometric analysis

Histological analysis showed normal kidney structure in the saline and harmine treatment groups. After treatment with morphine, 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 morphine + harmine at all doses, reduced the kidney damage caused by morphine toxicity (Fig. 2). Morphometric analysis revealed that morphine significantly increased the mean diameter of the glomerulus tubule and decreased the glomeruli number compared to the

saline group ($P < 0.05$). Treatment with harmine significantly increased the diameter of the glomeruli ($P < 0.05$) and no significant in the number of glomeruli in all treatment groups compared to the saline group ($P > 0.05$). Treatment with harmine + morphine significantly decreased the number of glomeruli ($P < 0.05$) and no significant in the diameter of the glomeruli in all treatment groups compared to the saline group ($P > 0.05$). Treatment with harmine and morphine + harmine significantly decreased the diameter of the glomeruli and increased the number of glomeruli in all treatment groups compared to the morphine group ($P < 0.05$) (Fig. 3).

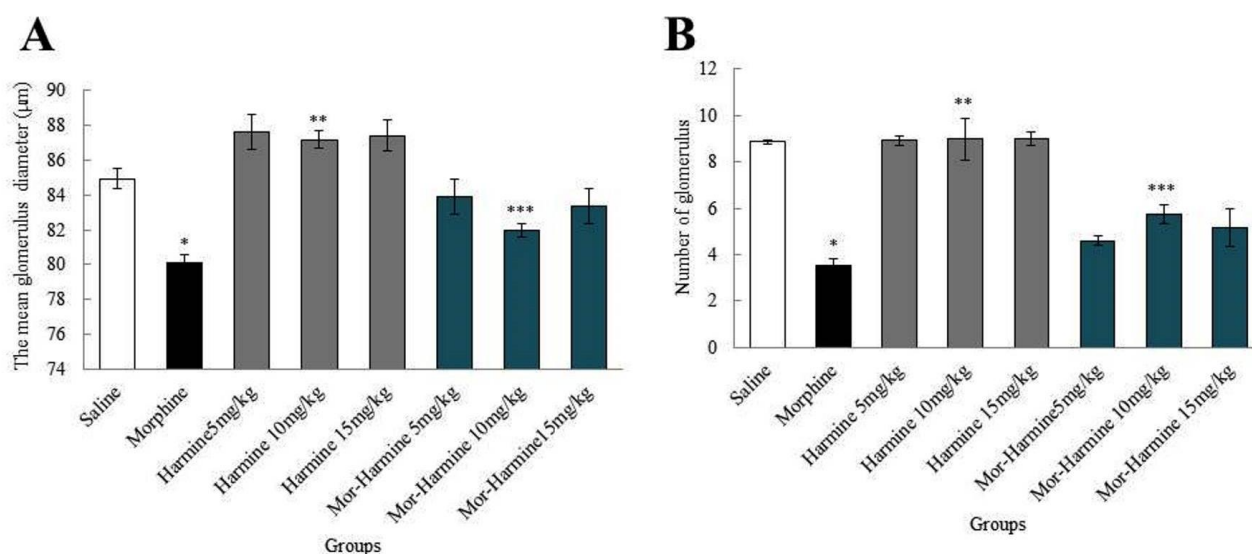


Figure 3. Correlation between treatment groups for (A) glomerular diameter; (B) glomeruli number. *Significant increase in morphine group compared to saline group ($p < 0.05$). **Significant increase for all harmine groups compared to morphine and saline (in glomerular diameter) groups ($p < 0.05$). ***Significant increase (compared to morphine group) and decrease (compared to saline group) for all harmine + morphine groups ($p < 0.05$).

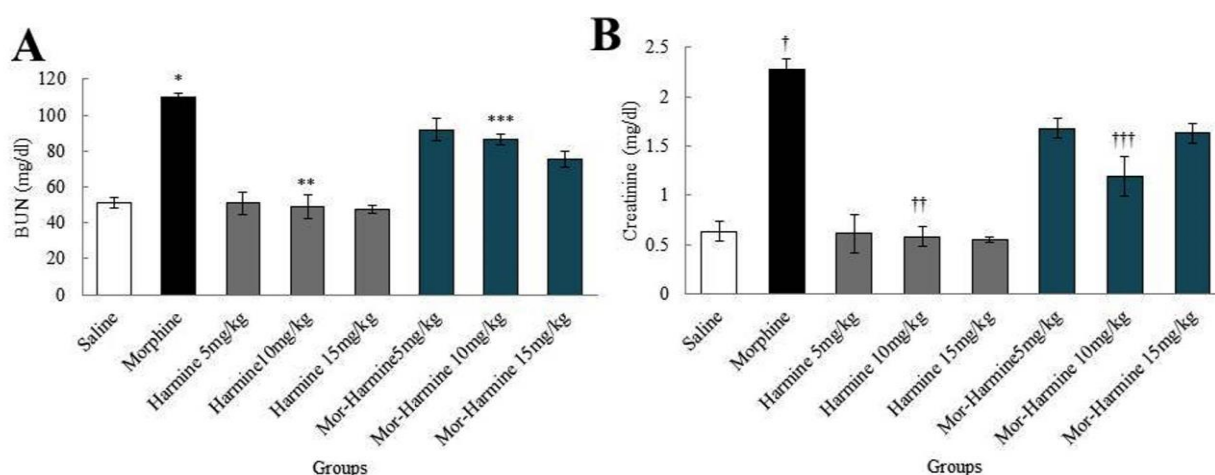


Figure 4. Effect of morphine, harmine and harmine + morphine on the mean kidney biochemical factors: (A) BUN (B) creatinine. *Significant increase in biochemical factor in morphine groups compared to saline groups ($P < 0.05$). **Significant decrease in biochemical factor for all of harmine groups compared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

Biochemical marker

Morphine (2.5 ml/kg) significantly increased the mean plasma BUN and creatinine concentration compared to the saline group ($P < 0.05$). The mean plasma BUN and creatinine concentration no significant in all harmine groups compared to the saline group ($P > 0.05$). The mean plasma BUN and creatinine concentration increased significantly in all harmine + morphine groups compared to the saline group ($P < 0.05$). The mean plasma BUN and creatinine concentration decreased significantly in all harmine and harmine + morphine groups compared to the morphine group ($P < 0.05$)

(Fig. 4).

Nitrite oxide

The mean nitrite oxide in the blood serum increased significantly in the morphine (2.5 ml/kg) group compared to the saline group ($P < 0.05$). The mean nitrite oxide in the blood serum no significant in all harmine and harmine + morphine groups compared to the saline group ($P > 0.05$). The mean nitrite oxide in the blood serum decreased significantly in all harmine and harmine + morphine groups compared to the morphine group ($P < 0.05$) (Fig. 5).

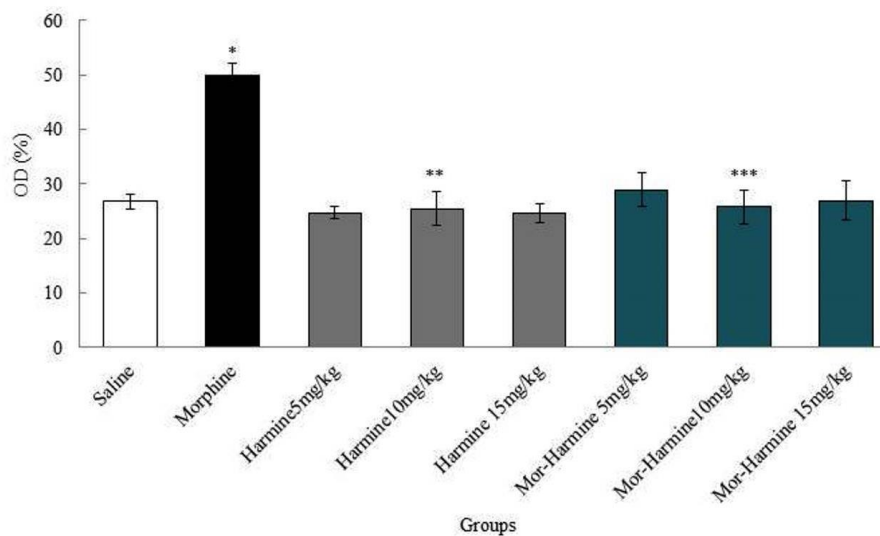


Figure 5. Effects of harmine, morphine and harmine + morphine on mean nitrite oxide levels. *Significant increase in nitrite oxide in morphine group compared to saline group ($P < 0.05$). **Significant decrease for all harmine groups compared to morphine group ($P < 0.05$). ***Significant decrease for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

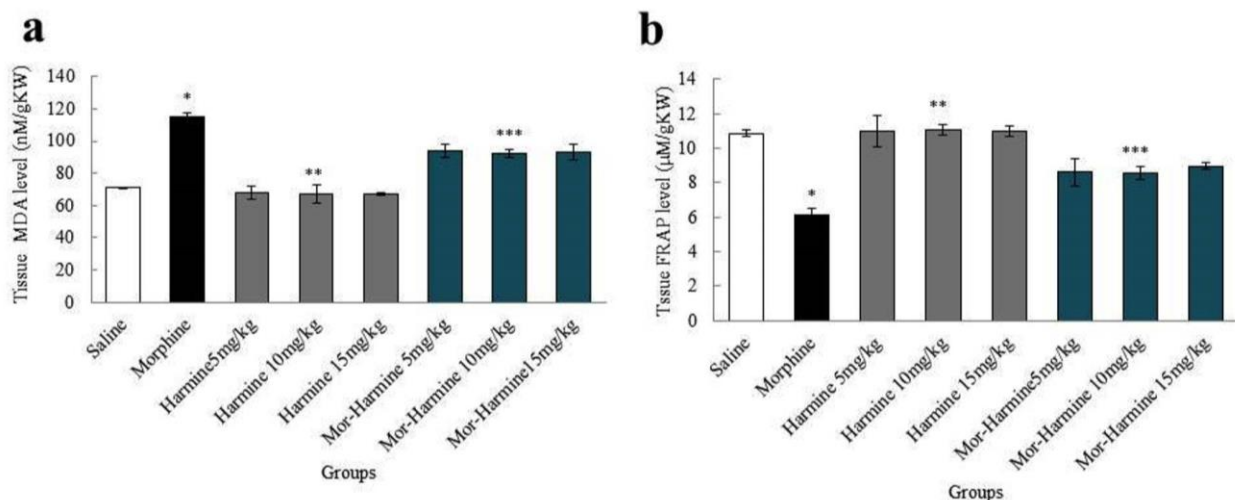


Figure 6. Comparison of morphine, saline and harmine + morphine groups of: (a) kidney MDA level; (b) tissue FRAP level. *Significant increase in morphine group compared to saline group ($p < 0.05$). **Significant decrease for all harmine groups compared to morphine group ($p < 0.05$). ***Significant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

Oxidative Stress

The results of the oxidative Stress testing in the groups showed that the kidney MDA level significantly increased in the morphine group compared to the saline group ($P < 0.05$). Harmine decreased significantly the kidney MDA level in all treatment groups compared to the morphine group ($P < 0.05$). The kidney MDA level decreased significantly in all harmine + morphine groups compared to the morphine group ($P < 0.05$). Similarly, morphine significantly decreased the renal tissue FRAP level of the morphine 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 + morphine groups compared to the morphine group ($P < 0.05$). Treatment with harmine in all groups, no significant in the renal tissue FRAPS and kidney MDA levels compared to the saline group ($p > 0.05$). Treatment with harmine + morphine significantly increased the kidney MDA level ($P < 0.05$) and no significant in the renal tissue FRAPS level in all treatment groups compared to the saline group ($P > 0.05$) (Fig. 6).

DISCUSSION

Chronic kidney disease is a general health problem. Morphine is an analgesic that is used clinically to alleviate severe pains and plays a key role in the pathogenesis of kidney disease [22]. Harmine is an herbal alkaloid from the betacarboline family which is extracted from *P. harmala* and different pharmacological effects [14]. The current study evaluated the effects of harmine on disorders induced by morphine in rats. The results, showed a significant decrease in the weight of the kidney and the whole body of morphine-receiving rats compared to the saline group. In the harmine + morphine groups, there was a significant increase in the weight of the kidney and whole body of the rats compared to the morphine group. It appears that morphine releases dopamine, serotonin and γ -amino butyric acid, suppresses the appetite and increases metabolism [23]. Arany et al. showed that prescribing morphine to rats with a high-fat diet significantly decreased their body weight and BMI. This agrees with the results of the current study [24]. It appears that morphine causes metabolism-induced generation of free radicals, lipid peroxidation, reacts with DNA and membrane proteins and causes cell damage as the main cause of weight and kidney index loss [1]. The results of Hamden et al. agree the findings of the current 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, morphine alone increased the diameter of the glomeruli and decreased the number of glomeruli. In groups treated with morphine + harmine, the diameter of the glomeruli decreased and the number of the glomeruli increased significantly compared to groups receiving only morphine. It appears that kidney glomeruli are very sensitive to oxidative stress (6). Morphine 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 morphine 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]. 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]. Jalili et al. reported that an increased concentration of cytosol calcium due to oxidative stress-induced mitochondrial damage in the cellular skeleton and interfered with the metabolism of mitochondrial energy by activating proteases, endonucleases and phospholipases, resulting 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 morphine for the number and diameter of glomeruli in this study [20] where the sole prescription of morphine increased BUN and creatinine in the blood serum of the study groups. In the morphine + harmine-receiving group, there was a significant decrease in the creatinine and BUN levels compared to the morphine 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]. Osborne et al. showed that morphine 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, it appears that harmine can reduce the effect of morphine 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 morphine group compared to saline group. In this study, the prescription of harmine + morphine significantly decreased the nitrite oxide level in the study groups. It appears that morphine 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 rats treated by rotenone [35]. The results of the current study reveal that harmine as

an antioxidant can lead to the relative mitigation of morphine-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 NF-K β expression. The current study consequences similarly displayed that treatment by harmine is able to moderate lipid peroxidation and increase anti-oxidant capacity of renal tissue, consequently reduces oxidative stress. Therefore, it seems that anti-oxidant properties of harmine via preventing the creation of reactive oxygen species could increase FRAPS and decrease MDA In the studied groups.

CONCLUSION

The results of this study indicate that harmine may recover some kidney function in rats treated with morphine. It could be valuable for protection of the kidney in individuals who have been exposed to morphine. Harmine antioxidant properties perhaps a 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

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

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