IJKD KIDNEY DISEASES

Protective Effect of Thymoquinone Against Morphine Injuries to Kidneys of Mice

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Original Paper

Introduction. Thymoquinone is a phytochemical compound found in the plant *Nigella sativa*. It has various pharmacological effects such as antioxidant and anti-apoptotic. Morphine can increase the generation of free radicals. It is mainly excreted through the kidneys and causes disturbing effects. This study was designed to evaluate protective effects of thymoquinone against morphine-induced damages to the kidneys of mice.

Materials and Methods. Various doses of thymoquinone (4.5 mg/kg, 9 mg/kg, and 18 mg/kg) were intraperitoneally administered along with morphine to 48 male mice for 20 consequent days. These mice were compared with a control group with saline injection, morphine group, and groups with same doses of thymoquinone only (n = 6 in each group). Blood urea nitrogen, serum creatinine, and serum nitric oxide levels, as well kidney weight and histology were assessed after the interventions.

Results. Morphine administration significantly decreased kidney weight and the number and mean diameter of the glomeruli. Increased levels of blood urea nitrogen, serum creatinine, and serum nitric oxide were also noted with morphine compared to the control group ($P < .05$). However, administration of thymoquinone and thymoquinone plus morphine significantly enhanced kidney weight, number and mean diameter of the glomeruli. All of the groups with thymoquinone were also associated with reduced blood urea nitrogen, serum creatinine, and serum nitric oxide levels compared to the morphine group ($P < .05$).

Conclusions. It seems that antioxidant and anti-apoptotic effects of thymoquinone could protect of the kidneys against damage due to morphine toxicity.

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INTRODUCTION

Study of compounds with plant origin is a very interesting branch of medical sciences. Many of these compounds have preventive effects, and as drugs with potentially fewer side effects and numerous properties, can be used to prevent certain diseases.[1] This property is often due to the presence of antioxidants in the plants, which prevent injuries associated with the free radicals.[2]

The extract of thymoquinone has anticonvulsant and antioxidant properties and is able to collect free radicals.[3] Thymoquinone, scientifically known as *Nigella sativa* L, is a family of *Ranunculaceae* with white or pale blue color, which is turned black when exposed to air.[4] *Nigella sativa* has antioxidative,

antibacterial, antihistaminic, antihypertension, antitumor, and anti-inflammatory properties, and it reduces blood glucose and relaxes the smooth muscles.[5] Thymoquinone, dithymoquinone, thymohydroquinone, and thymol are the main components of the hydroalcoholic extract of *Nigella sativa*.[6]

The study of Houghton and coworkers showed that administration of thymoquinone in the mice with allergic encephalomyelitis increased glutathione in the body, which is indicative of its anti-inflammatory and anti-allergic effects.[7] Further, the findings of Yaman and colleagues indicated that administration of thymoquinone oil in the mice consuming cyclosporine improved the performance of histological indexes of the kidney and inhibited the nephrotoxic effects of gentamycin.[8]

Morphine is an analgesic that is clinically used to relieve severe pains.[9] Morphine is a white or light brown crystal that is extracted from opium or directly obtained from the poppy stem.[10] Morphine metabolites are expelled through the kidneys.[11] This drug is biotransformed in the liver and kidneys and can damage the kidneys and liver.[12] This effect seems to be primarily induced by reduction of kidney plasma flow and increased secretion of urine reducing hormones (antidiuretics).[13] Morphine is closely associated with increased free radicals (through activating lipid peroxidation) and oxidative stress, which can lead to structural and functional disorders in body tissues.[14] Free radicals can cause cell membrane destruction and DNA segmentation.[15] A part of morphine, after absorption, is connected to the plasma proteins and is accumulated in tissues such as liver and kidney.[16] Morphine causes an increase in microinjection of podocytes in the kidneys, weakens the immune system, induces hypoxia, stimulates diuretic-releasing hormones from the kidneys, and causes vascular vasodilation.[17] Given the antitoxic effects of morphine and its abundant use as an analgesic in medicine, on the one hand, and thymoquinone properties, especially antioxidative properties, on the other hand, and since no study has investigated the protective effects of thymoquinone on morphine injuries to the kidneys, the current study was conducted to evaluate the protective effects of thymoquinone on kidney dysfunction due to the injuries induced by morphine in the male mice.

MATERIALS AND METHODS

Animals

A total of 48 balb/c male mice, with the body weight range of 27 g to 30 g, were used in this study. The animals were purchased from Razi Institute, Iran. They were kept in the animal house under laboratory conditions at 20 ± 2°C and 12-hour light cycle, with free access to usual water and food. The animals were kept in standard cages of the animal house of the medical school, each 6 mice in 1 cage. Maintenance and care of experimental animals complied with the National Institutes of Health guidelines.[18] Experiments were designed to conform to the International Guiding Principles for Biomedical Research Involving Animals (1985).

Experimental Protocol

The mice were randomly divided into 8 groups (n = 6), in order to receive: (1) normal saline, 1 mL/d (control); (2) morphine; (3) thymoquinone, 4.5 mg/kg; (4) thymoquinone, 9 mg/kg; (5) thymoquinone, 18 mg/kg; (6) morphine plus thymoquinone, 4.5 mg/kg; (7) morphine plus thymoquinone, 9 mg/kg; and (8) morphine plus thymoquinone, 18 mg/kg. Morphine was administered intraperitoneally as follows: 20 mg/kg, once daily, within the first 5 days; twice per day within the next 5 days; and a dose of up to 30 mg/kg, twice per day, on days 11 to 20. [19] Thymoquinone was administered intraperitoneally, once daily on days 1 to 20.[20] Mice with morphine plus thymoquinone received thymoquinone once daily on days 1 to 20, and morphine on days 19 and 20. The same volume of saline was administered in the control group.

Morphine (C16H19NO3) and thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone; C10H12O2) were obtained from Sigma Chemical (St Louis, USA) and were dissolved in saline (0.9%) for administration.

Blood Samples and Kidney Specimens

The day following the last injection, the mice of each group were consecutively placed in a closed plastic container, containing ether-soaked cotton to be anesthetized. Blood samples were taken from the heart using a 5-mL syringe. The blood samples were then incubated at 37°C for 15 minutes to coagulate. The coagulated blood was then centrifuged at 3000 rpm for 15 minutes to isolate its serum. The isolated serum was preserved at -20°C until the

measurement of kidney enzymes and nitric oxide. Animals were killed, the abdomen was opened by an incision on the white line followed by another behind the last rib to expose the abdominal organs and both kidneys were removed. The perinephric fat and renal fascia were removed in all the specimens and the kidneys were weighted on a microbalance sensitive to 0.001 mg (Precisa 125A, Switzerland) and average weights of the kidneys of rats were calculated and recorded.[2]

Histological and Morphometric Examinations

The kidney tissue was studied in terms of glomerulus diameter and number, leukocyte infiltration, and renal vein enlargement. The specimens were washed in saline and were fixed in 10% formalin at room temperature for 72 hours. After fixing the tissue, it was thoroughly washed under running water, dehydrated in ascending concentration ethanol according to a standard method, and cleared in xylene. Also, paraffin wax embedding procedures were used. Cuts of 5 µm were obtained (Leica RM 2125, Leica Microsystems Nussloch, Germany). From each sample, 5 sections (5, 8, 11, 14, and 17) were selected for analysis in order to avoid cell recounting. Following hematoxylin-eosin staining and preparing microscopic slides, 3 fields of view were selected from each slide for analysis and then subjected to analysis using an Olympus BX-51T-32E01 research microscope connected to a DP12 Camera with 3.34-million pixel resolution and Olysia Bio software (Olympus Optical, Tokyo, Japan). For each glomerulus, the total glomerulus area was measured (the distance between the basal membranes of the Bowman capsule to the corresponding point). The outline of each glomerulus was measured after taking an image with a 40× objective. The longest and shortest axes were measured in the drawing of each glomerulus in order to estimate the mean diameter (mean axis). From each animal, 20 samples of each structure were studied, totaling 120 structures per group.[21]

Biochemical Analysis

Serum and urine creatinine were photometrically measured without removing protein by Humastar 600 analyzer and commercial kits (Pars Azmoon, Tehran, Iran) (coefficients of variation = 2.38%) using Jaffe method. Serum urea nitrogen was measured by enzymatic UV method using Humastar 600 analyzer and laboratory kits (coefficients of variation =3.3%).[22]

Nitric oxide Measurement

Nitric oxide measurement was carried out by estimating its sustainable metabolite, nitrate, by Grice reaction using microplate method. In sum, 6 mg zinc sulphate powder was mixed with 400 µL serum in microtube and vortexed for 1 minute. After mixing, the samples were centrifuged at 10000 rpm at 4°C for 10 minutes, and supernatant was used for nitrate measurement. To recover nitrate to nitrite, vanadium chloride recovery (III) method was used, and serum nitrite level (µmol/L) was calculated by the Grice method.[23]

Statistical Analysis

All the quantitative data were presented as mean ± standard deviation. The 1-way analysis of variance was performed, followed by the LSD post hoc test, to determine the statistical significance between different groups using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA). A P less than .05 was considered significant.

RESULTS

Weight of Kidney

Morphine administration caused a significant decrease in the kidney weight of the mice compared to the control group ($P < .05$). Moreover, kidney weight was significantly increased in the animals treated with thymoquinone and thymoquinone plus morphine at all doses in comparison with the morphine group ($P < .05$; Figure 1).

Morphometric Measurements

The mean diameter and number of glomeruli were significantly decreased in the morphine group in comparison with the control group ($P < .05$). Furthermore, thymoquinone and thymoquinone plus morphine caused a significant increase in the mean diameter of glomeruli and number of glomeruli in all treated groups in comparison with group treated with morphine ($P < .05$; Figure 2).

Histopathological Observations

Histological examination showed normal kidney structure in the control group. After 20 days of

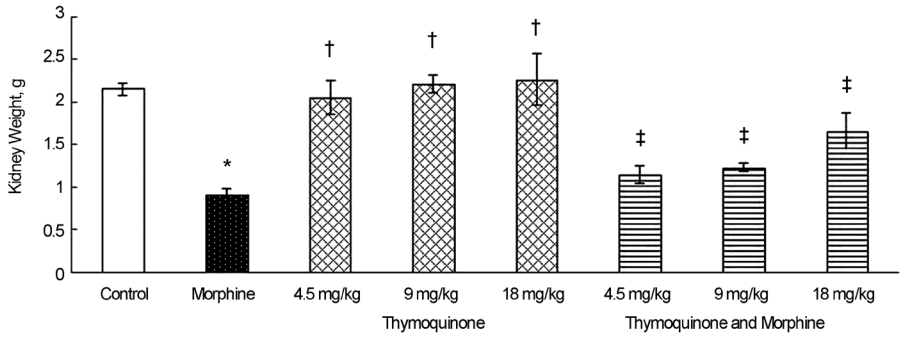

Figure 1. Kidney weight in mice receiving morphine and thymoquinone.
*P < .05 compared with the control group
†P < .05 compared with the morphine group
‡P < .05 compared with the morphine group

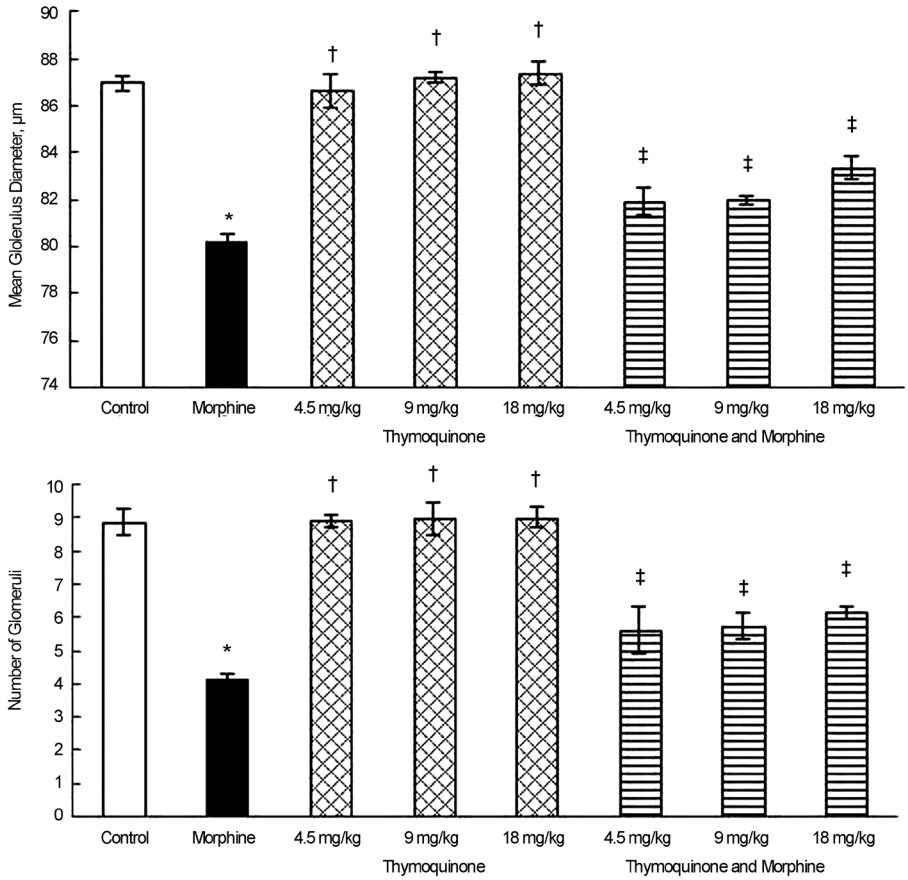

Figure 2. Glomeruli characteristics in mice receiving morphine and thymoquinone.
*P < .05 compared with the control group
†P < .05 compared with the morphine group
‡P < .05 compared with the morphine group

treatment with morphine, the kidney section appeared with variable changes and marked injury. These changes were evident by increased infiltration of leucocytes, decreased diameter of glomeruli, and enlarged kidney veins compared to the specimen from the control group. After 20 days of treatment with thymoquinone, 18 mg/kg, the kidney section indicated normal histology. After treatment with morphine plus thymoquinone, 18 mg/kg, it was recognized that thymoquinone reduced kidney injury caused by morphine toxicity and largely suppressed lymphocytic infiltration (Figure 3).

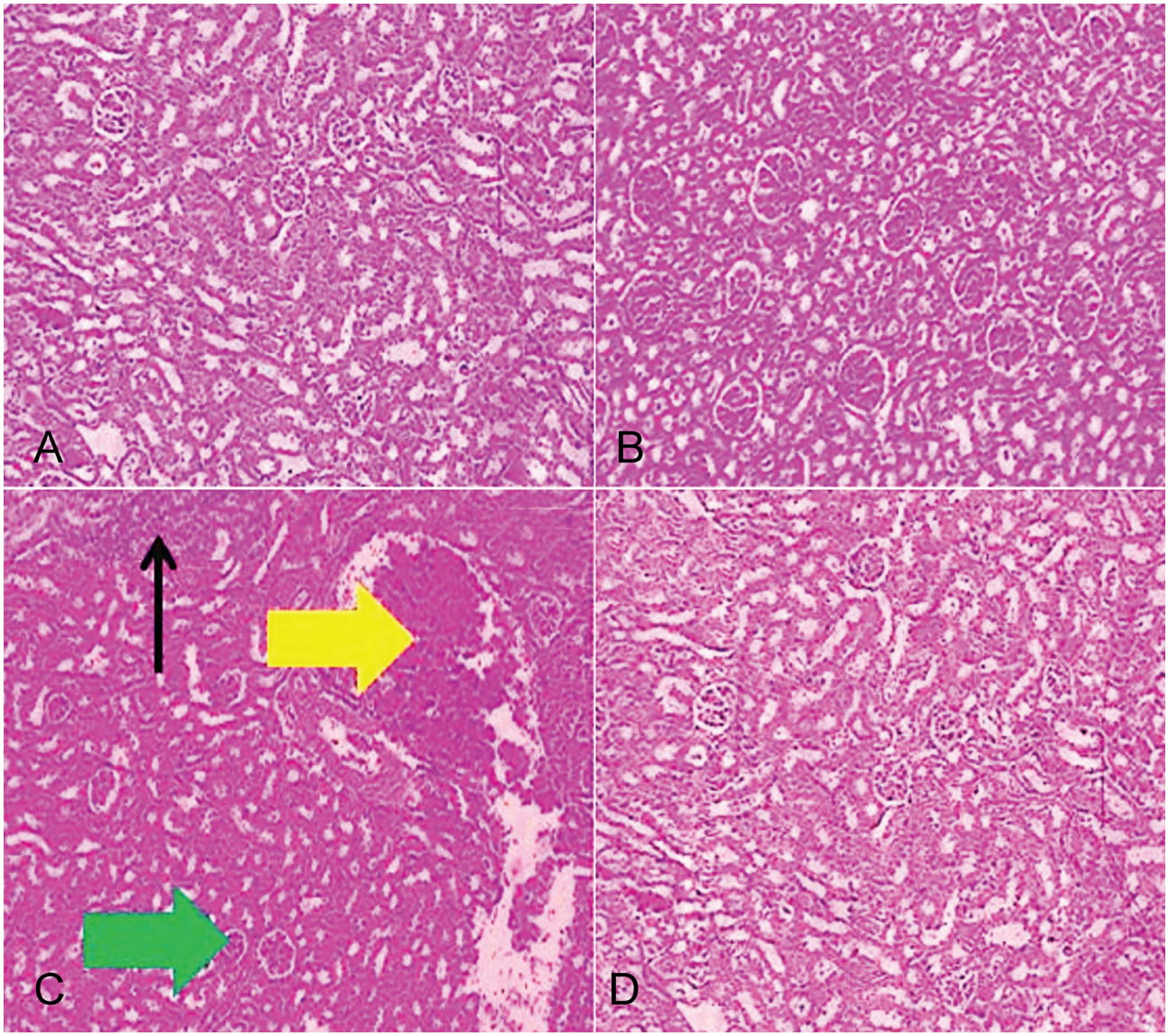

Figure 3. Histological changes of the kidneys (hematoxylin-eosin, × 100). A, Normal kidney structure in the control group. B, Normal kidney structure in the group treated with thymoquinone, 18 mg/kg. C, More distributed leucocytes (thin arrow), decrease the diameter of glomeruli (green thick arrow), and enlargement of kidney veins (yellow thick arrow) in the morphine group. D, Normal kidney structure in the group treated with morphine plus thymoquinone, 18 mg/kg.

Biochemical Analysis

Morphine caused a significant increase in creatinine and urea in serum compared to the control group ($P < .05$). In addition, the mean creatinine and blood urea nitrogen decreased significantly in the thymoquinone and thymoquinone plus morphine groups compared to the morphine group ($P < .05$; Figure 4).

Nitric Oxide

The mean nitric oxide levels in serum increased significantly in the morphine group compared to the control group ($P < .05$). Also, serum nitric oxide levels decreased significantly in the thymoquinone and thymoquinone plus morphine groups compared to the morphine group (Figure 5).

DISCUSSION

Kidneys are one of the most significant organs that regulate hemostasis and play a role in expelling the toxins and waste materials resulting from metabolism. Opioids are metabolized in the liver and their metabolites are excreted through the kidneys. This can be one of the possible reasons for

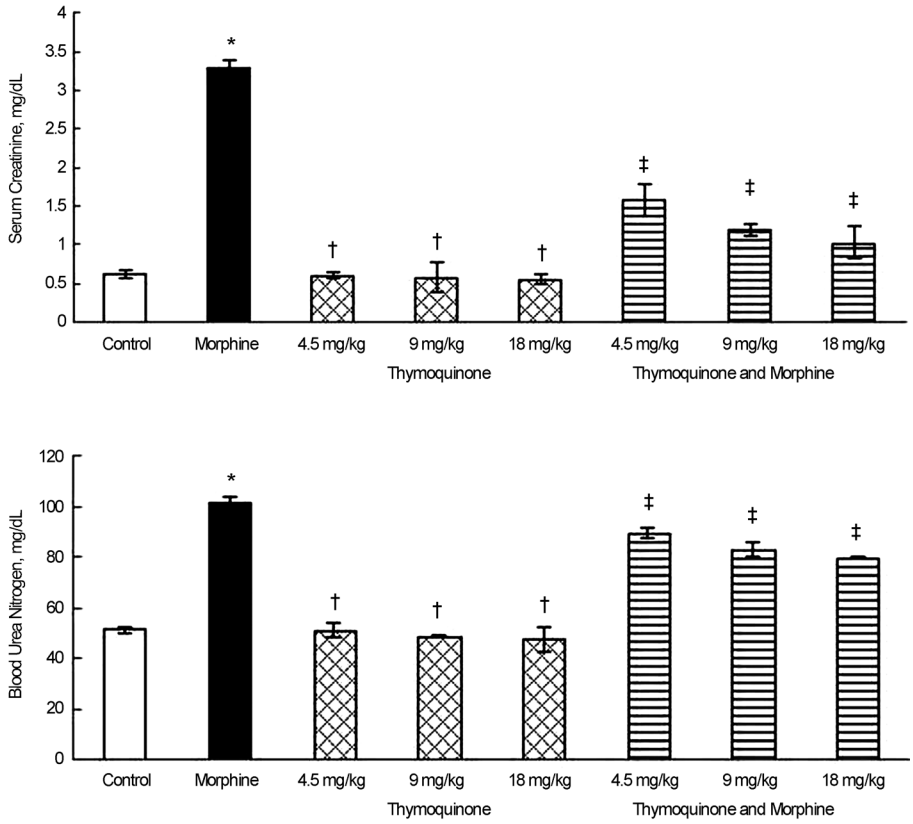

Figure 4. Kidney function markers in mice receiving morphine and thymoquinone.
*P < .05 compared with the control group
†P < .05 compared with the morphine group
‡P < .05 compared with the morphine group

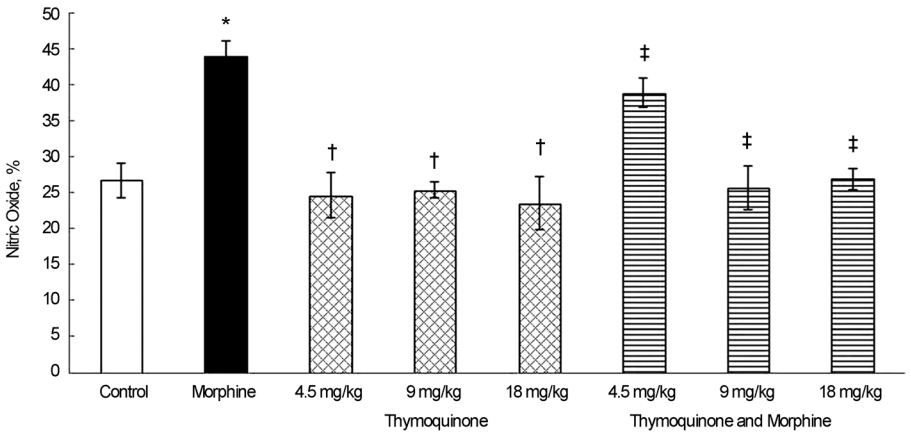

Figure 5. Nitric oxide level in mice receiving morphine and thymoquinone.
*P < .05 compared with the control group
†P < .05 compared with the morphine group
‡P < .05 compared with the morphine group

the pathologic effects of opioids on the kidneys.[13] The amount of reactive oxygen species in the cells and tissues in normal conditions, owing to a balance between their production and removal by the antioxidant defense system, remains constant to a certain limit. Morphine, through extensive production of free radicals, can change this balance in favor of production of reactive oxygen species and induce oxidative stress. In the present study, administration of morphine reduced the number and

diameter of renal glomeruli; whereas, simultaneous administration of thymoquinone and morphine reduced the effects of morphine on the kidneys.

Glomeruli are specific units of the kidney with similar size and form that are highly important in the quantitative analysis of kidney filtration function and are specifically sensitive to oxidative stress. It seems that the reduced size of glomeruli in the group receiving morphine can be indicative of the pathologic effects of morphine on glomeruli and primary pathological changes of the kidney.[24] Chemicals and their active metabolic forms may be transferred from plasma to renal tubules, where they accumulate. Complications of toxicity of some compounds distort the ion balance, causing the secretion of materials like phosphorus and calcium into urine or loss of the required materials, which consequently result in changes in the cells and wall thickness and reduced diameter of renal tubules.[25] Therefore, it is possible that any dysfunction in the flow of channels are associated with regulation of renal metabolic activity; particularly sodium channels can cause renal atrophy.

Morphine induces the proliferation of mesenchymal cells through opioid receptors, which is followed by kidney dysfunction. Studies have shown that morphine stimulates the mitogenic signals of the kidney in vivo. Since the filtration level of glomeruli depends on the number, mean diameter and structural consistency of these components, reduction of the number and mean diameter of glomeruli can be accompanied by functional disorders of the kidney.[26] Following the oxidative stress induced by administration of morphine, the activity of endonuclease enzymes is increased, thereby causing DNA damage through internucleosomal fragmentation of cells.[27] In addition, oxidative stress can cause protein dysfunction and cell apoptosis activation via affecting the mitochondria.[28]

Thymoquinone, as a potent antioxidant, can decrease oxidative stress by inducing glutathione and inhibitory effect on P450 cytochrome and prevent further metabolism of morphine, thereby reducing the production of free radicals.[29] Also, the study of Mahmoud and colleagues indicated that administration of thymoquinone could induce protective effects on the kidneys, through expression of Bcl-2, which is an anti-apoptotic factor.[30] The study carried out by Elbarbry and colleague showed that thymoquinone could reduce the toxic effects of drug metabolism via reduction of oxidative stress in the liver,[29] which confirms the results of the current study(29). The results of the analysis of the mean weight of the kidneys between the studied groups revealed a significant reduction in the mean weight of kidneys between the morphine and saline groups. Furthermore, it was found out that thymoquinone could partially inhibit the effects of morphine on renal weight reduction in the studied groups. Since morphine administration seems to impair the kidneys and create complications in the renal metabolism of the mice, it can reduce the weight of kidneys, too.[31]

The increased weight of the kidneys can be indicative of the nutritional improvement of the mice under treatment with thymoquinone. This increase can be a factor indicating the impact of thymoquinone on the enhanced nutrition of the studied animals.[32] Jalili and colleagues demonstrated that crocin could act as an antioxidant to induce protective effects against the toxicity due to morphine administration on the reduced weight of kidneys, which is in line with the findings of the current study.[15]

Urea and creatinine increase (creatinine is not subjected to renal reabsorption and secretion after filtration) in serum shows glomerular damage, which can be due to reduced renal excretion of these materials.[33] The results of this study indicated that administration of thymoquinone improved the performance of kidneys following the toxicity caused by morphine and reduction of serum urea and creatinine levels. Stimulation and activation of nuclear factor kappa B by oxidative stress can induce inflammatory activity in mesenchymal cells. On the other hand, it seems that the protective effects of thymoquinone on decreasing serum creatinine and urea levels are associated with its role in down regulation of nuclear factor kappa B. Furthermore, Sharma and associates showed that thymoquinone reduced the expression of nuclear factor kappa B in nephrotoxicity caused by cisplatin, which confirms the results of the present study.[34] The study conducted by Badary indicated that thymoquinone can significantly reduce urea, triglyceride, and cholesterol levels in oxidative stress-induced nephropathy in mice.[35] Findings of the current study showed that thymoquinone can, to some extent, inhibit the effects of morphine

on increasing nitric oxide in blood serum. Nitric oxide is a free radical whose increased production is accompanied by various diseases.[36] Morphine can increase nitric oxide production through intracellular regulation of calcium and activation of calcium/calmodulin-dependent nitric oxide synthases as well as naloxone-sensitive receptors.[37] Moreover, antioxidants damage nitric oxide system (protein enzymes, substrates, and cofactors), thereby reducing nitric oxide production. In line with the results of the present study, Nagi and colleagues showed that the protective effects of thymoquinone inhibited the production and synthesis of nitric oxide and production of superoxide radicals owing to its antioxidant properties.[38]

CONCLUSIONS

According to the results of this study, administration of thymoquinone seems to reduce the cellular and histological impairments resulting from the toxicity of morphine in the kidneys and to exert protective effects on the kidney of the mice through various ways such as prevention of oxidative stress, regulation of metabolic activities of kidney, and acting as an anti-apoptotic factor. Furthermore, the findings showed thymoquinone decreased nitric oxide, creatinine, and urea levels of serum, which are increased as a result of morphine administration, indicating the protective effects of thymoquinone on the performance of kidneys.

CONFLICT OF INTEREST

None declared.

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