Anti-urolithiatic activity of *Solanum nigrum* hydroalcoholic extract in ethylene glycol-induced urolithiasis in rats

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Received: 2 April 2019
Accepted: 21 April 2019

Egyptian Pharmaceutical Journal 2019, 18:311–319

Background and objectives
Urolithiasis is a growing public health problem. Asymptomatic kidney stones are kept under observation. The aim of this study was to explore the anti-urolithiatic activity of the hydroalcoholic extract of *Solanum nigrum* fruit in ethylene glycol-induced urolithiasis in rats.
Materials and methods
Urolithiasis was induced by oral administration of ammonium chloride 1% and ethylene glycol (0.75% v/v) in drinking water for 28 days. Hydroalcoholic extract of *Solanum nigrum* fruit (200 and 400 mg/kg) and cystone (750 mg/kg) were administered orally from the 15th day as a curative regimen.
Results and conclusion
Administration of ethylene glycol caused an elevation of serum creatinine, urea, calcium, and malondialdehyde and a reduction of magnesium and glutathione. In addition, renal content of tumor necrosis factor alpha was elevated and adiponectin renal content was reduced in urolithiatic control. Histopathological examination revealed tubular degeneration, dilatation, presence of calcium oxalate crystals in the lumen of renal tubules, and intense interstitial mononuclear cell infiltration in the lithiatic control group. Treatment with both doses of *Solanum nigrum* reversed all biochemical parameters and histopathological alterations. The results demonstrate that the hydroalcoholic extract of *Solanum nigrum has* potent anti-urolithiatic activity against calcium oxalate urolithiasis induced by ethylene glycol through tumor necrosis factor alpha inhibition and adiponectin stimulation as well as in maintaining balance between stone promoter (calcium) and inhibitor (magnesium).

Keywords:
adiponectin, ethylene glycol, rat, *Solanum nigrum*, urolithiasis

Egypt Pharmaceut J 18:311–319

1687-4315

Introduction

Urolithiasis can be defined as the formation of crystalline and matrix materials aggregation within the urinary tract [1]. Urolithiasis is provoked by decreased urine volume; increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate; reduction in fluid intake; increased exercise with dehydration, and medications leading to the formation of urinary calculi [2].

The worldwide prevalence of kidney stone formation has elevated owing to a change in diet with high lipid intake, in the last quarter of the 20th century [3]. Moreover, the prevalence of hypercholesterolemia, hypertriglyceridemia, and obesity is believed to be a predisposing factor for kidney stones [4].

Inflammation is an organism's natural defense response to stimuli, such as chemical substances like ethylene glycol which causes nephropathy associated with intrarenal calcium oxalate (CaOx) deposition and renal inflammation in experimental animal studies [5]. A proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), is gaining importance in urological practice [6]. It is used as a marker to predict renal parenchymal damage.

Kidney stone disease is associated with metabolic syndrome components, including obesity, hypertension, and diabetes mellitus. Metabolic syndrome is linked with high insulin resistance, which elevates the risk for kidney stone formation. In contrast, adiponectin, a circulating adipokine, might protect against kidney stones development [7]. Adiponectin administration ameliorated several diseases such as diabetes, nephropathy, and renal fibrosis [8].

Urinary or kidney stones are associated with high recurrence rate [9]. There are various modalities of treatment, such as a medical and surgical approach and

shock-wave lithotripsy, which depends upon stone location and obstruction extent [10]. These treatments induced adverse effects such as tubular necrosis and fibrosis of the kidney, leading to cell injury and recurrence of renal stone formation [11]. From the aforementioned facts, there is a great demand to investigate herbal plants with higher efficacy and lesser adverse effects for the treatment of urinary stones in the developed world.

Solanum nigrum is commonly called black night shade that belongs to Solanaceae (potato) family. Its bioactive ingredients, such as alkaloids, solanins, saponins, flavonoids, tannins, steroidal glycoalkaloids, steroidal genin, and vitamins, are responsible for diverse activities, including anti-inflammatory, antioxidant, nephroprotective, and cytoprotective effects [12].

Here, we evaluated the possible effects of *Solanum nigrum* hydroalcoholic extract and explored the possible mechanisms of amelioration of kidney crystal via inhibition of TNF-α and stimulation of adiponectin against ethylene glycol-induced urolithiasis in rats.

Materials and methods

Plant materials

Solanum nigrum fruit was collected from Agriculture Research Centre, Ministry of Agricultural Research Centre, Egypt.

Animals

Adult male albino rats of Wistar strain weighing 130–150 g, provided by National Research Centre, Egypt, were used throughout this study. The animals had free access to food and water *ad libitum* and maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12-h light and dark cycle.

Chemicals

Ethylene glycol and ammonium chloride were purchased from Sigma Aldrich Chemical Co. (St. Louis, Missouri, United States).

Methods

Preparation of plant extract

Solanum nigrum fruit was grinded and extracted with 70% aqueous ethanol at room temperature. The powder was soaked in 70% aqueous ethanol at room temperature overnight. The 70% aqueous ethanol extract was filtered using Whatman No. 1 filter paper. The filtrate was dried in rotavap under vacuum of 120mB in water bath at 40°C. The distillate was added on the marc, recollected again, and re-evaporated in the rotavap. Then, the extract was freeze dried (lyophilized) using freeze dryer to eliminate water traces. The extracts were then placed in glass vials and stored frozen at 20°C till being used.

Experimental design

Ethylene glycol (0.75%) and ammonium chloride (1%) were used for induction of renal calculi till the 28th day in drinking water, except the control group [13]. Animals were divided into five equal groups containing six animals each as follow: group I was the normal control, which received saline (5 ml/kg) for 28 days, group II was urolithiatic control, which received ethylene glycol (0.75%) and ammonium chloride (1%) for 28 days in drinking water, group III was the standard treated control, which received ethylene glycol (0.75%) and ammonium chloride (1%) in drinking water+cystone (750 mg/kg) from the 15th day till the 28th day [14], and groups IV and V received ethylene glycol (0.75%) and ammonium chloride (1%) in drinking water+*Solanum nigrum* extract (200 and 400 mg/kg), respectively, from the 15th day till the end of experimental period [15].

Serum biochemical analysis

Twenty-four hours following the last administration, blood samples were withdrawn from rats of all groups via retro-orbital vein under light ether anesthesia. Serum was used for estimation of serum creatinine (mg/dl), urea (mg/dl), calcium (mg/g), and magnesium (mg/24 h) using specific diagnostic kits (Biodiagnostic, Dokki, Giza, Egypt).

Biochemical analysis of renal tissue

Immediately after blood sampling, animals were killed by cervical dislocation under ether anesthesia. The two kidneys from each rat were immediately dissected out and rinsed with PBS to remove excess blood. Right kidneys from all groups were homogenized using (MPW-120 homogenizer; Med Instruments, Poland) in PBS to obtain 20% homogenate; the homogenates were centrifuged for 5 min at 5000*g* using a cooling centrifuge (Sigma and Laborzentrifugen, 2k15, Germany). The supernatant was removed immediately and assayed for reduced glutathione (GSH) (mg/g tissue) and lipid peroxides, measured as malondialdehyde (MDA) (nmol/g tissue), using Biodiagnostic Kits. TNF-α and adiponectin (pg/g tissue) were estimated using Elabscience Eliza Kits.

Histopathological examination

Kidney tissues from normal and other treated groups were removed and fixed in 10% neutral

formalin. Following routine processing, the tissue specimens were cut into 5-μm-thick sections and stained with hematoxylin and eosin for routine histopathological examinations. A semiquantitative grading score scaled from 0 to 3 was used to assess the renal damage relying on the percentage of tissue affected, in which 0=no histological abnormalities, 1=mild alterations (the lesions were demonstrated in <25% of tissue), 2=moderate alterations (25–75%), and 3=severe alterations (Ë 75%). The main histopathological parameters used for evaluation were tubular degeneration and/or necrosis, tubular dilatation, interstitial inflammation, and presence of oxalate crystals in the tubular lumina in addition to tubular regeneration. Ten fields per group were examined for assessment of renal damage.

Immunohistochemical analysis

The paraffin-embedded renal sections were dewaxed, rehydrated in graded alcohol, and incubated in 3% hydrogen peroxide to block endogenous peroxidase activity. Subsequently, sections were incubated with polyclonal antibodies specific for nuclear factor-κB (NF-κB). Finally, demonstration of immunoreactivity was carried out with diaminobenzidine (Sigma Aldrich Chemical Co.). Evaluation of NF-κB immune reactive cells was performed semiquantitatively according to the method of Hassan *et al*. [16]. Depending on the percentage of positive cells in the microscopic high-power field (HPF) (×40), the samples were scored as 0 (no staining), 1 (positive staining in <30% of cells per HPF), 2 (positive staining in 30–70% of cells per HPF), or 3 (positive staining in >70% of cells per HPF). A total of 10 random HPFs were used to estimate the percentage of positive cells.

Statistical analysis

All the values are presented as means±SE. Comparisons between different groups were carried out using one-way analysis of variance followed by Tukey's honest significant difference test for multiple comparisons.

Results

Effect of *Solanum nigrum* extract treatment on kidney functions

Administration of ethylene glycol showed a significant elevation in serum creatinine by 68.30% and serum urea by 1.74 fold, compared with those of normal group. Treatment of rats with high dose of *Solanum nigrum* extract and cystone decreased serum creatinine levels by 15.60 and 23.87%, respectively, compared with ethylene glycol group. In addition, treatment with high dose of *Solanum nigrum* extract and cystone normalized urea serum levels, compared with ethylene glycol group (Table 1).

Effect of *Solanum nigrum* extract treatment on serum calcium and magnesium levels

Results exhibited a significant elevation in calcium and reduction in magnesium serum levels by 14.62 and 19.64%, respectively, after administration of ethylene glycol as compared with those of control group. However; the treatment with both doses of *Solanum nigrum* extract showed the anti-urolithiatic activity by normalizing the level of calcium and magnesium levels, and also cystone treatment normalized serum calcium and magnesium levels, compared with ethylene glycol group (Table 1).

Effect of *Solanum nigrum* extract treatment on oxidative stress markers

Renal GSH content was significantly reduced by 31.05% following ethylene glycol administration, and renal MDA content was significantly elevated by 1.2 fold, as compared with those of the control group. However, renal GSH content significantly elevated following treatment with high dose of *Solanum nigrum* extract and cystone by 14.26 and 17.51%; on the contrary, normal levels of MDA were observed in groups treated with both doses of *Solanum nigrum* extract, whereas cystone treatment did not reach the normal level when compared with the normal group (Figs 1 and 2).

Effect of *Solanum nigrum* extract treatment on renal tumor necrosis factor alpha content

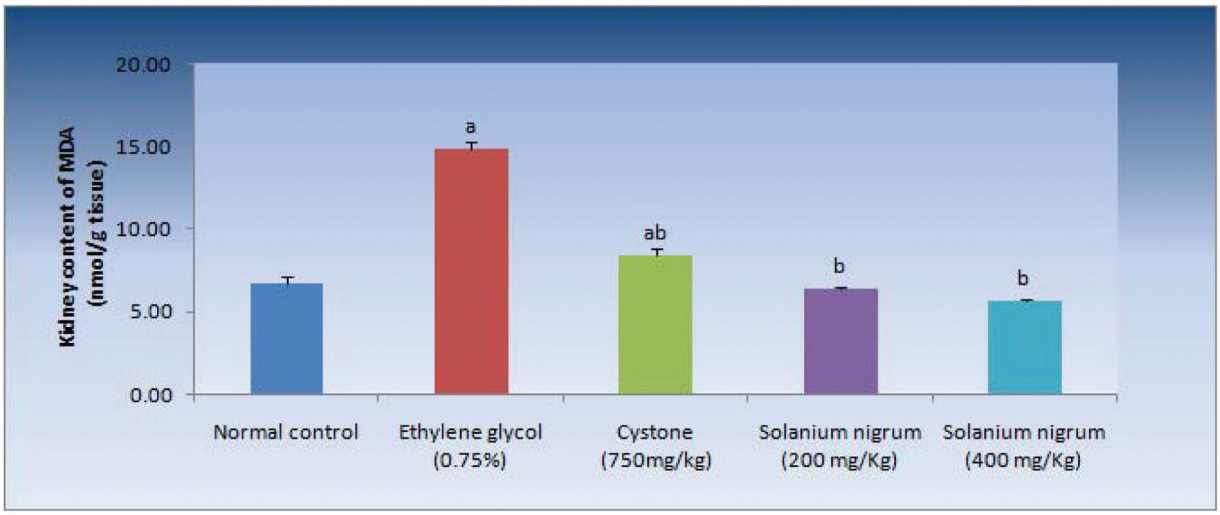
Induction of kidney stone by ethylene glycol significantly increased TNF-α renal content by

Table 1 Effect of *Solanum nigrum* extract treatment on serum creatinine, urea, calcium, and magnesium levels

	Normal control	Urolithic control	Cystone (750 mg/kg)	*Solanum nigrum* (200 mg/kg)	*Solanum nigrum* (400 mg/kg)
Creatinine (mg/dl)	0.71±0.02	1.20±0.06[a]	0.91±0.01[ab]	1.14±0.02[a]	1.01±0.01[ab]
Urea (mg/dl)	11.35±0.36	31.14±2.19[a]	10.69±0.08[b]	12.06±0.13[b]	15.33±1.50[b]
Ca (mg/g)	6.68±0.05	7.66±0.10[a]	6.71±0.03[b]	6.83±0.06[b]	6.76±0.03[b]
Mg (mg/24 h)	36.25±0.51	29.13±1.41[a]	34.47±0.21[b]	36.50±0.94[b]	38.03±0.85[b]

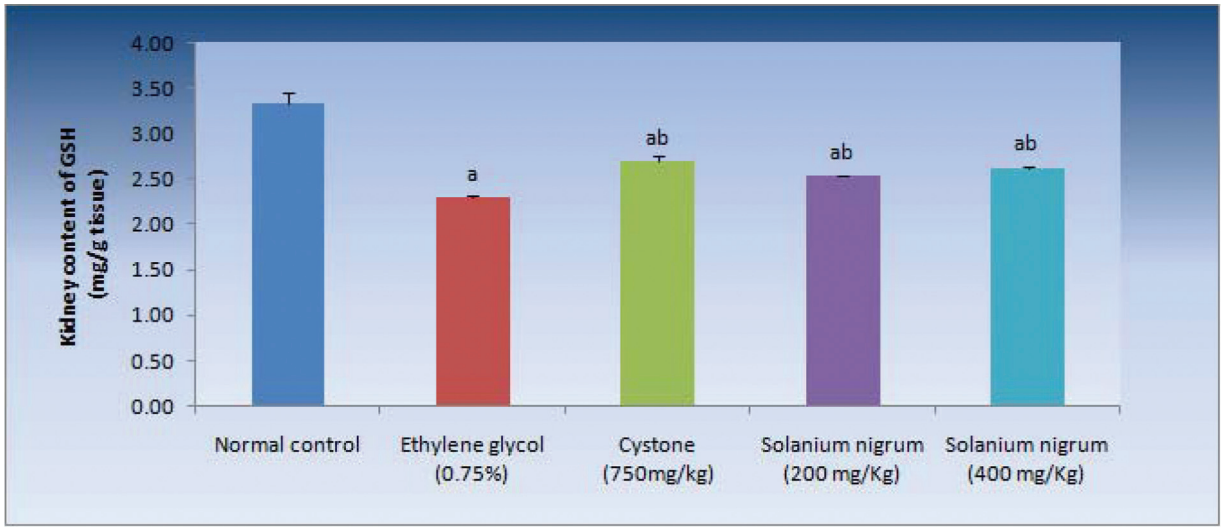
Data were expressed as mean±SE (n=6). Ca, calcium; Mg, magnesium. Statistical analysis was carried out by one-way analysis of variance followed by Tukey's honest significant difference test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from urolithic control at P value less than 0.05.

Figure 1



Effect of *Solanum nigrum* extract treatment on renal malondialdehyde (MDA) content. Data were expressed as mean±SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from urolithic control at P value less than 0.05. HSD, honest significant difference.

Figure 2



Effect of *Solanum nigrum* extract treatment on renal reduced glutathione (GSH) content. Data were expressed as mean±SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from urolithic control at P value less than 0.05. HSD, honest significant difference.

77.69%, as compared with normal control group. However, treatment with both dose of *Solanum nigrum* significantly decreased renal TNF-α contents by 18.39 and 27.07%, respectively, as compared with ethylene glycol group, whereas treatment with cystone showed normal renal content of TNF-α (Fig. 3).

Effect of *Solanum nigrum* extract treatment on renal adiponectin content

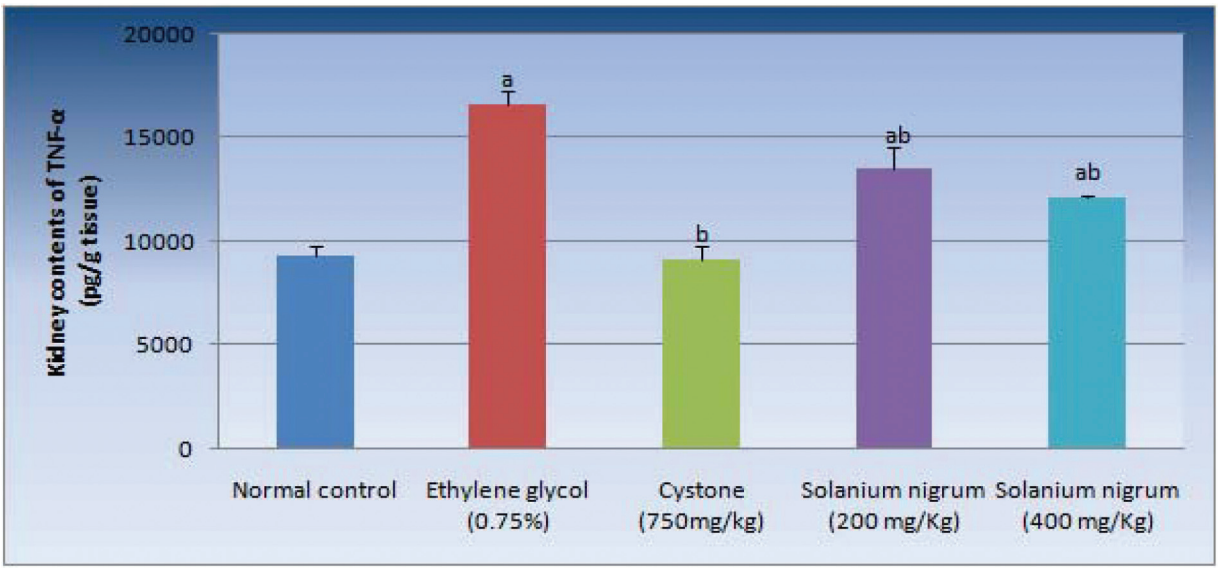
Serum adiponectin level was reduced in the ethylene glycol by 26.08% than in normal control group, whereas normal serum adiponectin levels were observed following treatment with high dose of *Solanum nigrum* and cystone as compared with ethylene glycol group (Fig. 4).

Histopathology

The mean pathologic renal damage estimated in the kidneys of normal and treated rats is illustrated in Table 2.

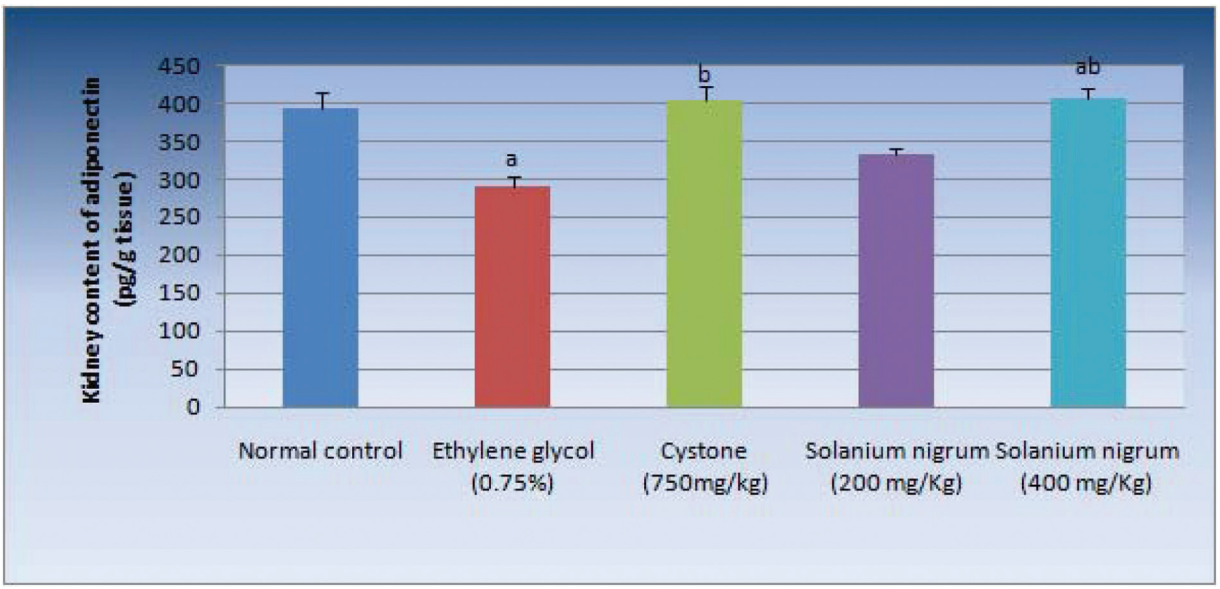
Kidney sections of normal rats showed normal histological structure with no definite histopathological lesions (Fig. 5a). In contrast, kidneys of urolithic control group revealed deleterious histopathological alterations represented

Figure 3



Effect of *Solanum nigrum* extract treatment on renal tumor necrosis factor alpha (TNF-α) content. Data were expressed as mean±SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from urolithic control at P value less than 0.05. HSD, honest significant difference.

Figure 4



Effect of *Solanum nigrum* extract treatment on renal adiponectin content. Data were expressed as mean±SE (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from urolithic control at P value less than 0.05. HSD, honest significant difference.

by extensive tubular dilatation associated with presence of abundant prominent refractile oxalate crystals in the lumen of the renal tubules which showed variable loss and attenuation of the epithelium (Fig. 5b) in addition to peritubular and perivascular leukocytic cell infiltration as well as presence of regenerative renal tubules. Regression of the histopathological lesions with pronounced amelioration was demonstrated in cystone-treated group, which revealed mild tubular dilatation and decreased inflammatory cellular infiltrates (Fig. 5c). Pretreatment with *Solanum nigrum* revealed improvement in a dose-dependent manner. Focal vacuolar degeneration of renal tubular epithelium associated with mild tubular dilatation was demonstrated in *Solanum nigrum* (200 mg/kg) group (Fig. 5d). On the contrary, mild granular degeneration

Table 2 The mean pathologic renal damage in the kidneys of normal and treated rats

Groups	Mean pathologic renal damage/field	NF-κB (% of positive cells/HPF)
Normal control	0.00±0.00[d]	0.00±0.00[c]
Urolithic control	2.90±0.10[a]	2.60±0.24[a]
Cystone	1.70±0.26[c]	0.80±0.20[b]
Solanum nigrum (200 mg/kg)	2.30±0.21[b]	2.00±0.31[a]
Solanum nigrum (400 mg/kg)	1.70±0.26[c]	1.20±0.20[b]

The mean pathologic renal damage estimated in the kidneys of normal and treated rats. HPF, high-power field; NF-κB, nuclear factor-κB. Different letters indicate significantly different means *P* value less than 0.05. Same letters indicate nonsignificant changes.

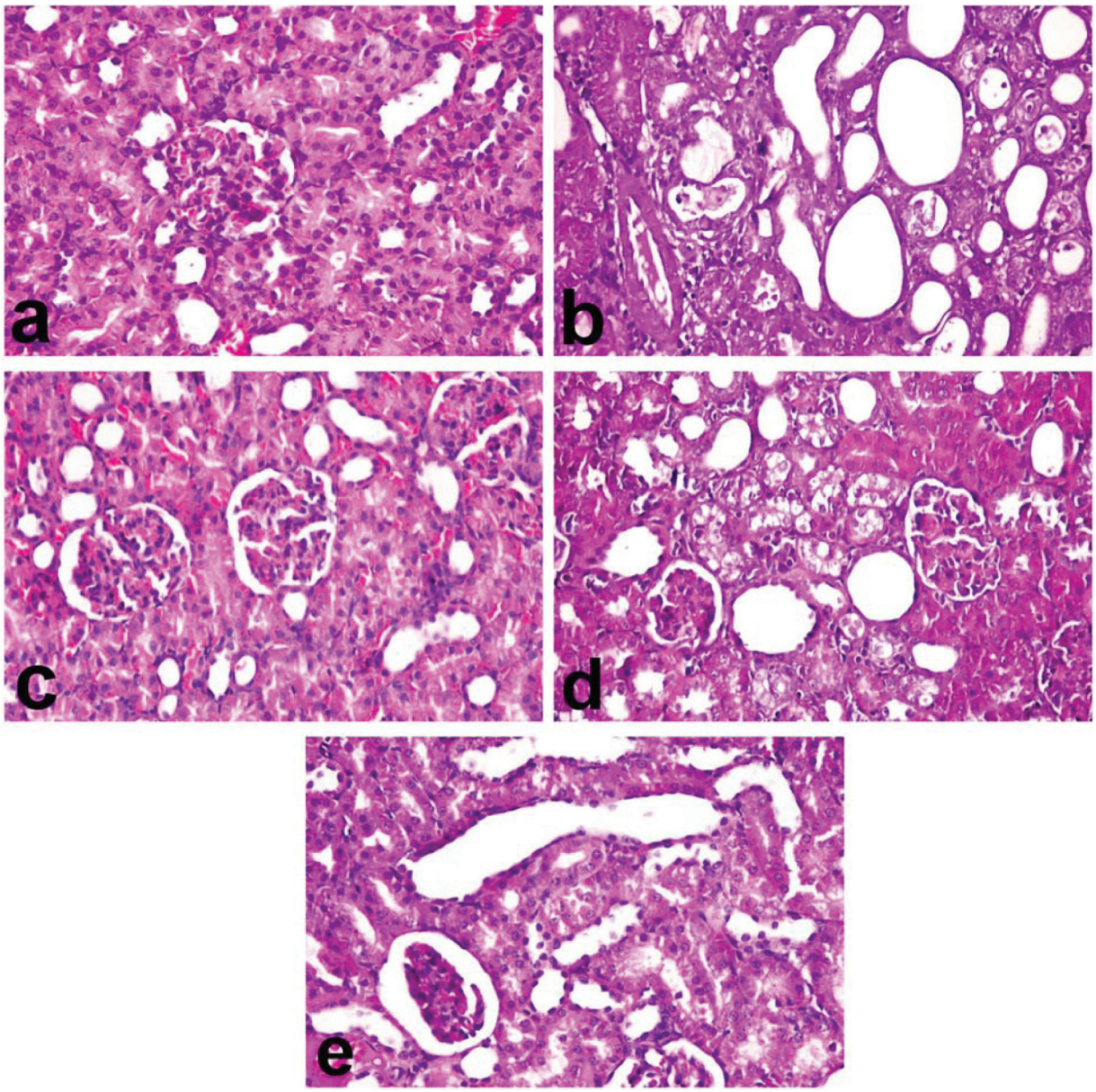
with minimal inflammatory infiltrates and tubular dilatation were observed in *Solanum nigrum* (400 mg/kg) group (Fig. 5e).

Immunohistochemistry

Table 2 demonstrates the mean percentage of NF-κB positively stained cells recorded in the kidney of normal and treated rats.

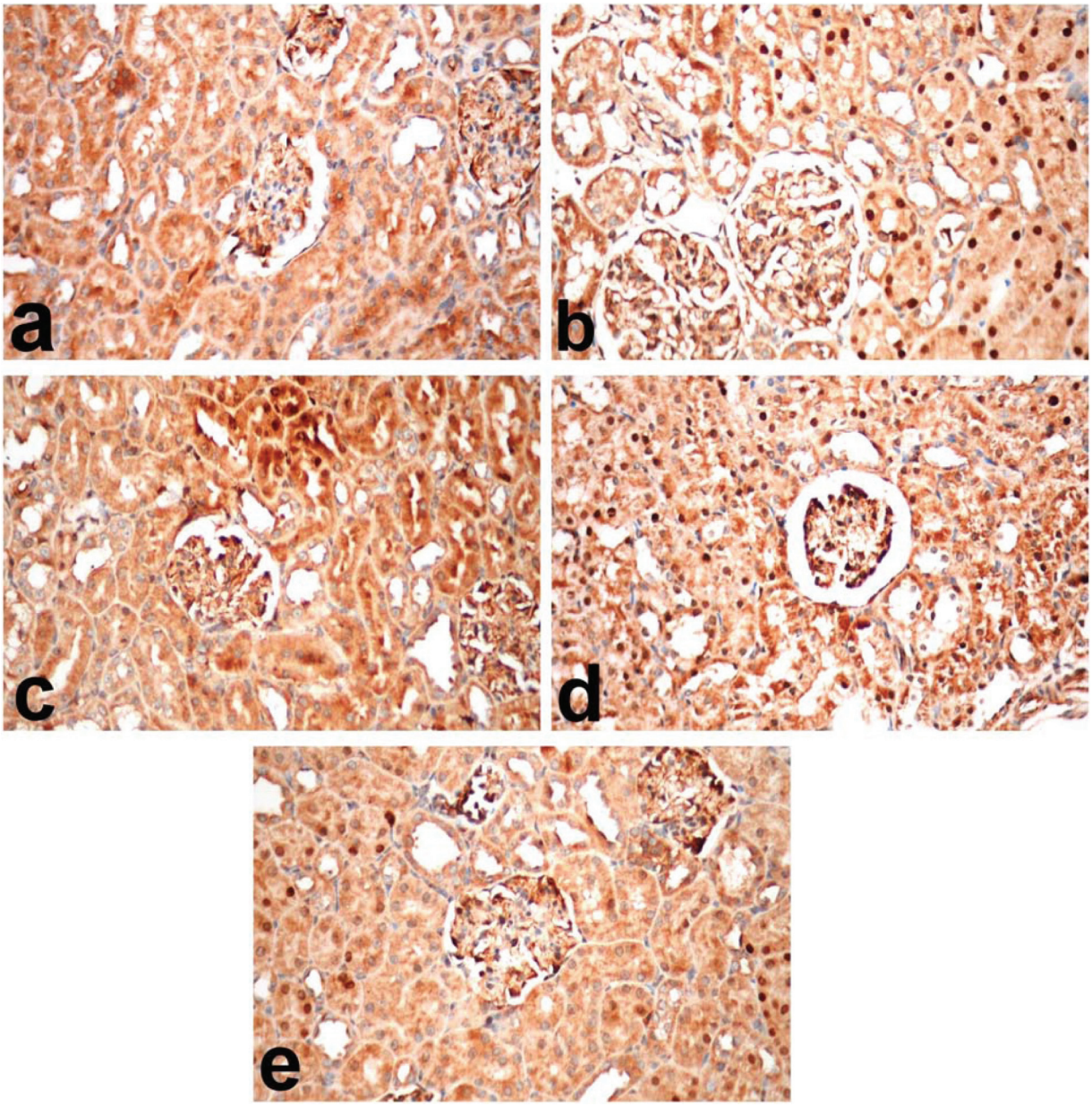
NF-κB immunohistochemical staining of kidneys of normal rats revealed cytoplasmic staining of renal tubular epithelium (Fig. 6a). Conversely, increased expression of NF-κb with increased percentage of

Figure 5



(a) Kidney of normal rats showing normal histological structures of glomeruli and tubules, (b) urolithic control rats showing extensive tubular dilatation associated with presence of abundant prominent refractile oxalate crystals in the lumen of the renal tubules, (c) cystone-treated rats showing mild tubular dilatation, (d) *Solanum nigrum* (200 mg/kg)-treated rats showing focal vacuolar degeneration of renal tubular epithelium associated with mild tubular dilatation, and (e) *Solanum nigrum* (400 mg/kg)-treated rats showing mild granular degeneration of renal tubular epithelium (hematoxylin and eosin, ×40).

Figure 6



(a) Kidney of normal rats showing cytoplasmic staining of renal tubular epithelium, (b) urolithic control rats showing increased expression of nuclear factor-κB (NF-κB) with nuclear translocation, (c) cystone-treated rats showing decreased number of positively stained nuclei, (d) *Solanum nigrum* (200 mg/kg)-treated rats showing increased number of positively stained nuclei, and (e) *Solanum nigrum* (400 mg/kg)-treated rats showing few scattered positively stained nuclei (NF-Òb immunohistochemical staining, ×40).

cells with nuclear translocation was demonstrated in the kidneys of urolithic control group, which revealed abundant intense brown nuclei (Fig. 6b). Marked reduction of expression with decreased percentage of positive cells with brown nuclear staining was recorded in cystone-treated group (Fig. 6c). On the contrary, increased percentage of positive cells with increased number of positively stained nuclei was recorded in *Solanum nigrum* (200 mg/kg) group (Fig. 6d), but few scattered positively stained nuclei were demonstrated in *Solanum nigrum* (400 mg/kg) group (Fig. 6e).

Discussion

In this study, ethylene glycol provoked stone formation and deposition of renal CaOx in the renal proximal tubular lumen located at the region between the renal cortex and the medulla, mimicking urinary stone formation in humans. Ethylene glycol, also, worsened kidney function by elevating creatinine and urea serum levels, exhibiting an increase in calcium serum level, and producing crystal growth and nucleation of CaOx in kidney, as shown in our histopathological study.

Treatment with *Solanum nigrum* improved kidney function through decreased creatinine and urea serum levels and exhibited a reduction in calcium serum level. Moreover, *Solanum nigrum* treatment ameliorated crystal aggregation and scattered the crystals throughout the entire kidney region as shown in our histopathological study. Similar results are also observed when methanol extract of *Solanum xanthocarpum* is used as an antilithic agent [17].

Stone formation is a consequence of an imbalance between promoters and inhibitors in the kidney [18]. The major components of kidney stones are inorganic materials, with a small percentage of organic material; 90% of the inorganic components are CaOx [19]. Magnesium is one of the inhibitors of crystallization, as encountered in ethylene glycol-induced stone formation, and it decreases the supersaturation of CaOx, reducing crystal growth [20]. In the current work, *Solanum nigrum* treatment restored the serum level of magnesium, reducing CaOx crystal growth. These results are supported by another study on *Solanum xanthocarpum* fruit extract that ameliorated nephrolithiasis in rats [17].

The stones induced obstruction of urine flow, thus reduced glomerular filtration rate, which is associated with waste products accumulation in blood [9]. Our work showed significant increase in creatinine and urea serum levels, which indicates glomerular and tubular damage. Treatment with *Solanum nigrum* improved glomerular filtration and prevented the elevation of creatinine and urea serum levels [21].

Hirose *et al*. [22] reported in the early phase of kidney crystal formation, a mitochondrial disorder induced oxidative stress and renal proximal tubular cell injury. Oxidative damage induced by ethylene glycol and ammonium chloride is reflected through the increased MDA and decreased activities of GSH in kidneys and deteriorated renal functions [23]. In our work, the reduction of renal GSH and the elevation of MDA contents are associated with proximal tubular cell injury observed in rats treated with ethylene glycol and ammonium chloride. However, treatment with *Solanum nigrum* preserved the functional capacity of renal GSH and prevented the elevation of MDA contents induced by ethylene glycol. In another study, *Solanum nigrum* restored the kidney catalase (CAT), glutathione peroxidase (GPx), and GSH against ethanol toxicity [24]. Moreover, Veerapagu *et al*. [25] reported higher total phenol and total flavonoid content in *Solanum nigrum* leaves that showed potent antioxidant activities [26].It was thought that crystals are associated with an elevation in TNF-α level from peripheral monocytes infiltrating the kidney [27]. Our results exhibited the elevation of TNF-α renal content with kidney crystal formation induced by ethylene glycol with ammonium chloride, whereas treatment with *Solanum nigrum* prevented this elevation of renal TNF-α level. In another study, *Solanum nigrum* leaves extract showed a significant decrease in TNF-α [28].

Adiponectin, a fat-derived hormone, is involved in kidney stone formation, which is a metabolic syndrome-related disease [29]. Adiponectin reversed insulin resistance [30] and ameliorated renal fibrosis in mice [8]. In our study, we showed the decrease of adiponectin renal content as a result of the progression of kidney crystal formation induced by ethylene glycol with ammonium chloride. In addition, we exhibited increased TNF-α in kidney content, and this might be the explanation of decreased adiponectin expression during kidney crystal formation. Fujii *et al*. [31] used adiponectin treatment to ameliorated crystal aggregation throughout the kidney region via TNF-α inhibition. For the first time, we showed that *Solanum nigrum* administration alleviated crystal aggregation through increasing renal content of adiponectin [32].

Conclusion

We can conclude from the aforementioned results that *Solanum nigrum* has renoprotective, anti-inflammatory, and antioxidative functions and might be an ideal curative medicine for kidney stones through downregulation of TNF-α and upregulation of adiponectin expression. These effects may be attributed to the presence of carbohydrates, alkaloids, triterpenoids, and flavonoids.

Acknowledgements

The authors acknowledge the NRC technical and financial support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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