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Antilithiatic effect of *Triticum aestivum* against sodium oxalate-induced lithiasis in rat model

Amany A. Sayed[1*]

Abstract

Background The present study pointed to evaluate the role of *Triticum aestivum* ethanolic extract (TAEE) in prophylactic and curative regimens on sodium oxalate (NaOx)-prompted lithiasis. Forty-eight rats were divided into the following regimen's group (24 rats/regimen). Groups I, II, III, and IV served as prophylactic groups and divided as: group I is a vehicle control received 5% DMSO (vehicle). All the remaining groups received NaOx (70 mg/kg b.wt; i.p.). Group II is NaOx lithiatic group, groups III and IV received TAEE and cystone prophylactically at doses of 500 and 750 mg/kg b.wt, respectively, since 1st day to 7th day of lithiasis stimulation. Groups V, VI, VII, and VIII served as curative groups that divided as the prophylactic ones but TAEE and cystone administered from 7th day to 14th day of lithiasis stimulation.

Results Lithiatic rats co-/post-treated with TAEE and cystone raised the urinary volume significantly. Also, TAEE showed modulatory effect in inhibiting and curative stone creation. Significant decreases were observed in the calcium and phosphate contents in urine or serum beside the increased magnesium value in lithiatic rats co-/post-treated with TAEE. TAEE significantly ameliorates the kidney function markers, which proposes its antilithiatic role. Moreover, lithiatic rats co-/post-treated with TAEE significantly diminished oxidative injury evoked by NaOx.

Conclusions TAEE has antilithiatic role that may be returned to its diuretic and antioxidant activity in addition to its bioactive components including ferulic acid and phytic acid that have the inhibitory properties on crystallization calcium oxalate by lowering the content of stone promotor constituents.

Keywords *Triticum aestivum* ethanolic extract, Lithiasis, Calcium oxalate, Oxidative stress

Background

Lithiasis (formation of stone) is a one of causative factors that induce acute or chronic renal failure and includes nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in urinary tract). Nephrolithiasis usually arise from an inequality between the requirement of kidneys to conserve body fluid and the necessity to remove the low solubility waste products, which resulted in disruption between calculi promoters and inhibitors (Duval et al., 2014; Pandhare et al., 2021). Lastly, this imbalance increased, which may be due to alterations in diet and fluid intake, degree of physical exercise and activity, and climate changes (Zarin et al., 2020). The prevalence of lithiasis increased globally, especially in the last decades, and now lithiasis is ranked as a third predominant complaint in the urinary system (Pawar & Vyawahare, 2017). Almost 80% of formed calculi are calcium oxalate (CaOx) or phosphate (Pandhare et al., 2021). Oxalate (Ox) is a chelating compound that causes a variety of pathological disorders, particularly CaOx nephrolithiasis (Deepika et al., 2013). Oxalate-induced

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renal membrane damage through lipid peroxidation process by formation of oxygen free radicals comprising superoxide anion and hydroxyl radicals (Ahmed et al., 2020). Jonassen et al. (2005) clarified that the interaction between oxalate and renal epithelial cells changed the properties of membrane surface, disrupted the mitochondrial function, and formed reactive oxygen species (ROS) and subsequently developed oxidative stress (OxS) that facilitates the growth of CaOx crystals to kidney stones. Therefore, previous studies select sodium oxalate (NaOx) as an appropriate source of oxalate for induction of nephrolithiasis (İlhan et al., 2014; Sayed et al., 2020; Takawale et al., 2012).

Currently, no satisfactory allopathic medicines are available for lithiasis in spite of a huge biological and physical studies to prevent lithiasis (Pandhare et al., 2021). However, the new techniques used for removal of calculi, for example extracorporeal shock wave lithotripsy (ESWL), lithotripsy and even laparoscopy high-power laser, are associated with serious complications, especially the recurrence of the stones (Pandhare et al., 2021). The repetition rate in lacking preventive management is about 10% after 1 year, 33% after 5 year and 50% during 10 years, which is the supreme central feature to be adopted (Basavaraj et al., 2007). Thereby, nephrolithiasis is not the only high prevalence disease that necessitates consideration, but it is one of the more challenging diseases due to its increased rate of reappearance after removal (Patel & Acharya, 2020). Thus, it is classified as one of the refractory diseases that require many investigations to find out the etiology of it and subsequently develop a proper therapy. Therefore, there is a need to replace these conventional therapies with a better medicinal plant or phytotherapy.

Medicinal herbs and plants have been exhausted as an substitute therapy for nephrolithiasis since ancient Egyptian era (Tefekli and Cezayirli, 2013). In recent, many investigators also moved toward medicinal natural agents for assessing their antilithiatic effectiveness [2]. The restoration to medicinal herbs may be due to their easy access, low cost, inherited experience, minimal side effects. Besides their ability to enhance the resistance of body against infection and promoting quick restoration of physiological functions (Sathya & Kokilavani, 2012).

Triticum aestivum L. is a cereal grass belonging to the family Gramineae. It is popularly called as wheat and cultured world widely, but it is local to South-West Asia and Mediterranean regions. *Triticum aestivum* is a crucial food for the world's population, particularly in developing countries. It is a safe herb and accepted in Food and Drug Administration screening and clearance (Durairaj et al., 2014). It is utilized as conventional herbal medication and is greatly promising for its therapeutic characteristics (Swati et al., 2010). The therapeutic abilities of *Triticum aestivum* backed to its health-enhancing chemical constituents, including dietary fiber, chlorophyll, enzymes, Vitamins (A, C, E, B1, B12), folic acid, phenolics, bioflavonoids, tocopherols, and 17 amino acids among them 8 are essential (Abdel-Aal & Hucl, 2003; Walters, 1992). Additionally, it contains many minerals including calcium, phosphorus, selenium, zinc, iodine, magnesium, boron, chromium, potassium, iron, and molybdenum (Sundaresan et al., 2015). Sekkoum et al. (2011) proved the in vitro antiurolithiatic activity of *Triticum aestivum*, even though no scientific data exist regarding the in vivo antilithiatic potential of the *T. aestivum*. To fulfill this limitation, the present study conducts a preclinical in vivo effectiveness of *Triticum aestivum* ethanolic extract (TAEE) on NaOx-induced nephrolithiasis in male rats in preventive and curative regimens. Cystone drug was taken as a standard antilithiatic for comparison of the results.

Methods

Preparation of *Triticum aestivum* extract

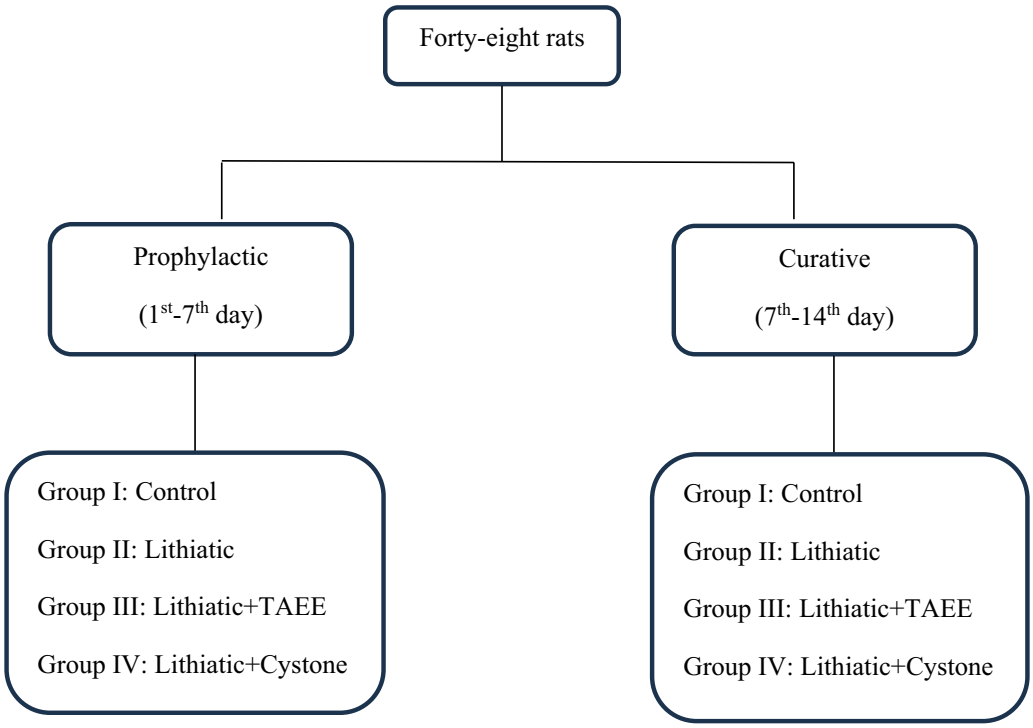
To prepare crude phyto-drug from *Triticum aestivum*, the present study selects ethanol as it is appropriate solvent to extract both polar and nonpolar chemical constituents from nutraceutical resources and to extract more phenolic contents (Kulkarni et al., 2006). The wheat cereals (*Triticum aestivum*) were powdered with a mechanical grinder and 400-g fine powder of *T. aestivum* was extracted with 600 ml ethanol at room temperature. Subsequent to 24 h, it was filtrated and the resultant TAEE (*Triticum aestivum* ethanolic extract) was inserted into rotary evaporator to concentrate to crude extract. The obtained extract was conserved in desiccator, at usage it suspended in dimethyl sulfoxide (DMSO, 5%) and treated orally by gavage to rats.

Animals

Forty-eight rats male Wistar albino rats (180–200 g) were obtained from National Research Center, Egypt. Prior to the experiment, rats were housed in a clean polypropylene cage (6 rats/each cage) with a stainless-steel cover grill that accommodate for pelleted food for 7 days for acclimatization of animal house conditions (23 ± 2 °C/50% humidity/12-h light: 12-h dark cycle).

Calculi induction

Intraperitoneal injection of 70 mg NaOx/kg b.wt induces lithiasis in 7 days (İlhan et al., 2014; Sayed et al., 2020). NaOx dissolved in physiological saline and is given alone for lithiatic induction or in combination with TAEE to evaluate its antilithiatic capacity.



Scheme 1 Schematic diagram showing the prophylactic and curative regimens of lithiatic rats

Experimental design

Animals were randomly alienated into eight groups each comprising six rats. Two types of regimens were accomplished: prophylactic study and curative study. Groups I, II, III, and IV served as prophylactic groups and divided as follows. Group I is a vehicle control; animals of this group received 5% DMSO (vehicle). All other remaining groups were treated with calculi-inducing agent (70 mg NaOx/kg, i.p.). Group II is NaOx lithiatic group, groups III and IV received TAEE and cystone prophylactically at doses of 500 and 750 mg/kg body weight, respectively, from first day to seventh day of lithiasis induction. Groups V, VI, VII, and VIII served as curative groups which divided as the preventive ones but TAEE and cystone administered from 7th day to 14th day of lithiasis induction. The standard drug (cystone) was suspended in saline. TAEE and the standard drug were given once daily by oral route via gastric gavage. All the prophylactic groups euthanized on the 8th day, while the curative ones euthanized on the 15th day (Scheme 1).

Animal handling

At the end of each regimen, rats were euthanized by sodium pentobarbital at overdose. Blood was collected in centrifuge tubes for different renal assays. Sera were separated by routine centrifugation method and stored at − 20 °C for assessment of renal function. Kidneys were quickly isolated, cleaned, and washed with saline to use for the oxidative/antioxidative assays and histological examination.

Assessment of urinary function parameters

One day before euthanizing the animals of both regimens, animals were housed alone to collect 24-h urine sample. Following measurement of urinary volume and pH, the urine samples were exposed to centrifugation to obtain supernatant. Microscopic analysis was done to examine the crystal formation. Then, investigation of calcium, phosphate, and magnesium beside kidney function markers was estimated using Bio-diagnostic kits (Dokki, Giza, Egypt). Animal was provided to drinking water but no feed during the urine collection period.

Assessment of serum kidney function biomarkers

After the experimental period, animals were euthanized and serum samples were obtained via blood centrifugation (10 min, 2500 rpm) to determine kidney function markers [creatinine (CAT. No. CR 12 50), uric acid (CAT. NO. UA 21 20), and urea (CAT. NO. UR 21 10)] according to Bartles et al. (1972) and Tietz et al. (1990), respectively. Other biochemical parameters such as calcium, phosphorus, and magnesium were determined according to Gindler and King (1972), El-Mezrabani et al. (1977), Grindler et al. (1971), respectively. All these parameters

were estimated according to the protocol of the prepared Bio-diagnostic kits.

Preparation of kidney tissue

Two kidneys were dissected out carefully, cleaned off surrounding extra tissues, washed in cold physiological saline and then used for estimation of oxidative/antioxidative parameters and histopathological examination.

Kidney homogenate analysis

The left kidney was minced and homogenized at 10% Tris–HCl buffer (0.1M, pH 7.4). The homogenate was centrifuged at 22,000 × *g* for 20 min at 4 °C to separate the nuclear debris and getting post-mitochondrial supernatant. Renal supernatant was used to analyze lipid peroxidation (LPO, CAT. No. MD 25 28) (Ohkawa et al., 1979), glutathione reduced (GSH, CAT. No. GR 25 10) (Beutler et al., 1963), superoxide dismutase (SOD, CAT. No. SD 25 20) (Nishikimi et al., 1972), catalase (CAT, CAT. No. CA 25 16) (Aebi, 1984), and glutathione- S- transferase (GST, CAT. No. GT 25 18) (Habig et al., 1974) according to the manufacture's protocols of Bio-diagnostic kits (Giza, Egypt).

Kidney histological examination

The right kidney was fixed in neutral buffered formalin (10%), dehydrated in a gradient series of ethanol, cleared in xylene, embedded in paraffin wax, stained with hematoxylin and eosin, and cross-sectioned to examine the renal architecture and scan calcium oxalate deposits under light microscope.

Statistical analysis

All results were displayed as mean ± standard error of mean (SEM) of six animals/each group. The statistical significance was assessed by ANOVA (one-way analysis of variance), and different groups were statistically compared by Duncan's test using SPSS software (the Statistical Package for the Social Sciences, version 15.0, SPSS Inc., Chicago, Illinois, USA) statistical package. Differences between groups (*P* value) were considered significant at $P < 0.05$.

Results

Effect of TAEE on urinary output and pH of lithiatic rats

The present study revealed that the urinary output of NaOx (lithiatic) group was significantly decreased ($P < 0.05$), as compared with control rats. However, lithiatic rats treated with TAEE (500 mg/kg b.wt) have increasing in urine volume ($P < 0.05$) significantly, as compared with untreated rats. It seems that TAEE has a diueitic effect either in prophylactic or curative therapies; as there is a significant increase ($P < 0.05$) in urinary output than the control rats also. Additionally, cystone caused elevated urinary volume of lithiatic rats significantly ($P < 0.05$). Neutral urinary pH was observed in control rats. On the initiation of lithiasis, pH become alkaline in untreated lithiatic rats as compared with the control ones. At the end of study, lithiatic groups co-treated with TAEE have acineutral urine, but TAEE restored urinary pH to neutral in curative regimen when compared to the respective untreated group (Table 1).

Morphological appearance of urinary crystals

Microscopic analysis of urine in both regimens revealed the absence of crystals in the control groups (Figs. 1A, 2A), whereas lithiatic groups have many crystals that appeared as CaOx (Figs. 1B, 2B). However, urine of TAEE exhibited less or detached crystal fragments with clearly reduced numbers and size (Figs. 1C, 2C). Similarly, cystone groups showed more or less low crystal appearance (Figs. 1D, 2D).

Effect of TAEE on urinary excretion markers of lithiatic rats

In prophylactic and curative regimens, a significant increase ($P < 0.05$) in urinary markers of kidney functions (creatinine, urea, and uric acid) was shown in the lithiatic groups in comparison with the respective control group. Urinary kidney function levels were reduced significantly ($P < 0.05$) after administration of lithiatic rats

Table 1 Effect of *Triticum aestivum* ethanolic extract (TAEE) on urinary output (volume) and pH of lithiatic rats

Parameters	Concurrent regimen				Curative regimen			
	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone
Urine volume (ml/24 h)	8.58 ± 0.74^{a}	2.40 ± 0.75^{b}	11.50 ± 0.818^{c}	9.70 ± 1.21^{ac}	9.33 ± 0.84^{a}	2.96 ± 0.18^{b}	16.50 ± 2.66^{c}	12.16 ± 1.81^{d}
pH of urine	Neutral	Slightly alkaline	Slightly neutral	Neutral	Neutral	Slightly alkaline	Neutral	Neutral

Values are expressed as mean ± SEM ($n = 6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P < 0.05$)

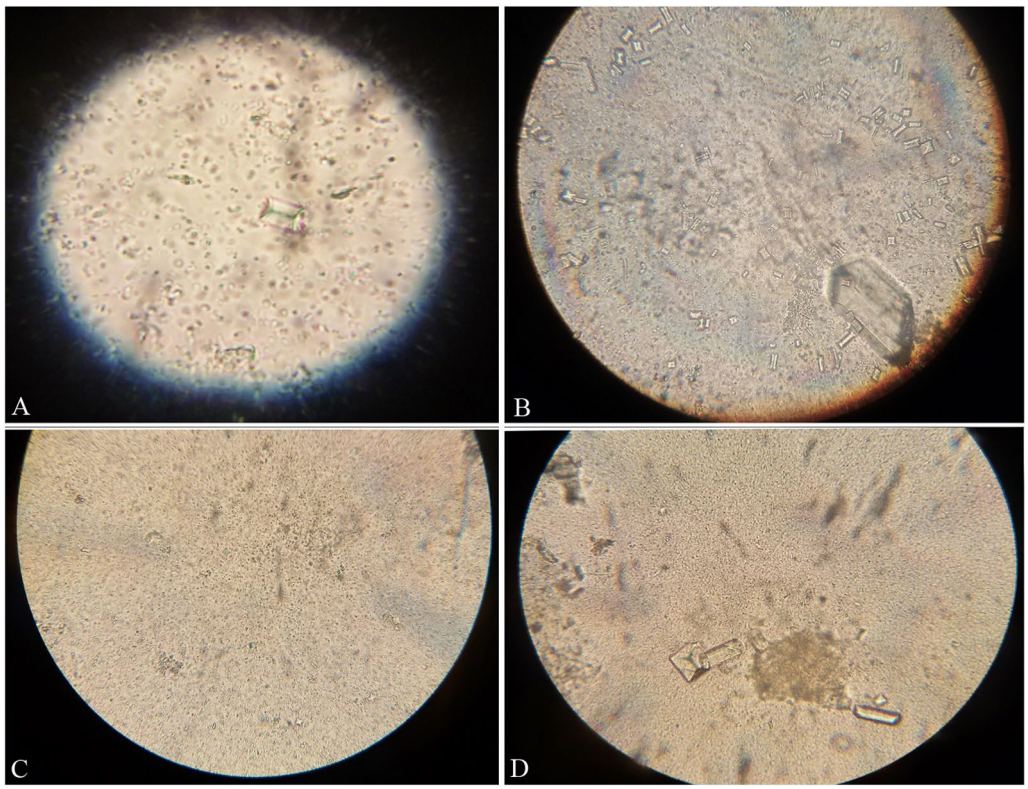


Fig. 1 Light microscopy of calcium oxalate crystals in urine in prophylactic group. **A** Control; **B** lithiatic; **C** TAEE; **D** cystone

with TAEE (500 mg/kg b.wt). Elimination of calcium and phosphate was significantly increased ($P<0.05$) in lithiatic rats, as compared with control. On the contrary, the urinary magnesium level was reduced in lithiatic rats, but this decrease was significant ($P<0.05$) only in the curative regimen relative to corresponding control levels. Supplementation with TAEE to lithiatic rats significantly ($P<0.05$) ameliorated the changed levels of each of calcium and phosphate as well as magnesium in comparison with corresponding untreated lithiatic rats. The modulatory results of TAEE were in line with cystone-treated rats (Table 2).

Effect of TAEE on serum kidney function markers of lithiatic rats

Injection of NaOx for 7 days caused significant rise ($P<0.05$) of serum markers of kidney functions in prophylactic and curative regimens in comparison with control levels. Further, significant elevation ($P<0.05$) was detected in serum calcium and phosphate levels either in prophylactic regimen or in curative one of NaOx group in comparison with the corresponding control levels. Conversely, serum magnesium level of lithiatic rats declined significantly ($P<0.05$) in both regimens as compared to corresponding control rats. These results point to more or less renal dysfunctions of urolithiatic rats. Administration of lithiatic rats with TAEE (500 mg/kg b.wt) significantly inverted ($P<0.05$) the serum modifications and restore the values nearly to normal value, when compared to lithiatic rats. Treatment with cystone significantly modulated ($P<0.05$) the concentrations of these markers, as compared with lithiatic rats. These findings provide a supportive indication of the similarity of the TAEE and the standard drug cystone (Table 3).

Effect of TAEE on kidney oxidative/antioxidative markers

In prophylactic and curative regimens, stone induction promotes the renal MDA level significantly ($P<0.05$) but significantly inhibited ($P<0.05$) GSH, GST, SOD, and catalase in corresponding untreated rats compared to control rats (Table 4). Remarkably, co- and post-administration of TAEE (500 mg/kg b.wt) to lithiatic rats inverted the oxidative/antioxidative variations significantly ($P<0.05$) comparative to the corresponding untreated ones. Also, cystone triggered significant improvement ($P<0.05$) in the oxidative and antioxidative status of untreated lithiatic rats.

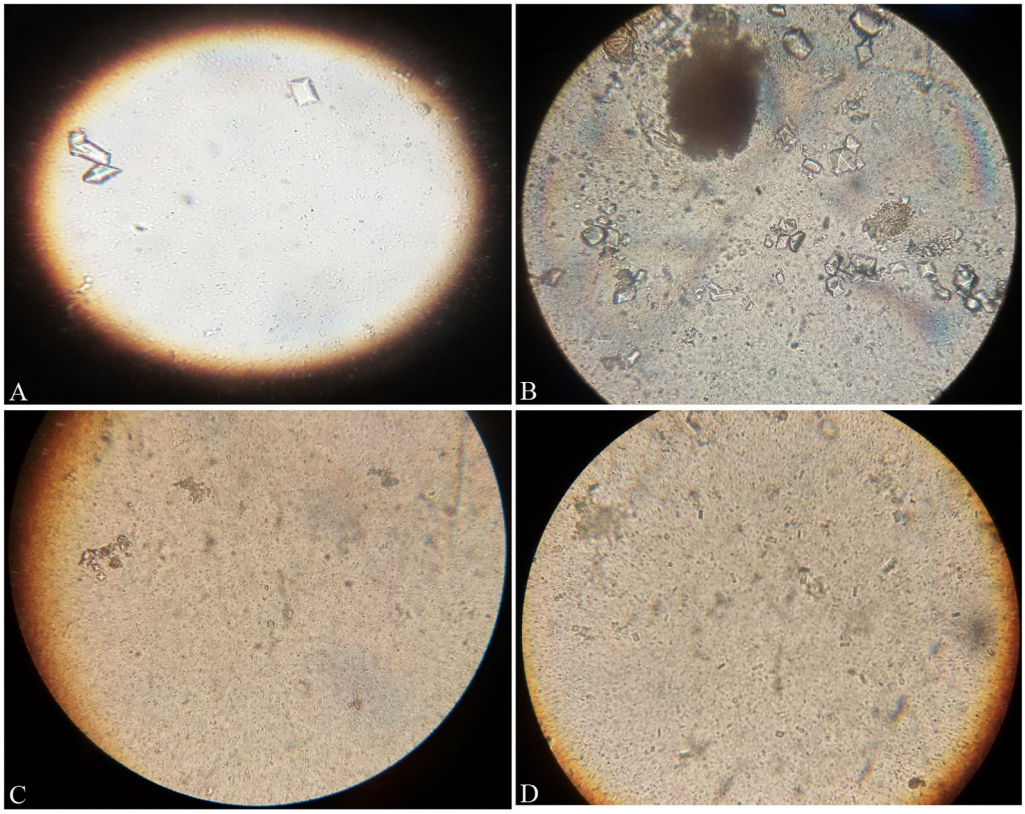


Fig. 2 Light microscopy of calcium oxalate crystals in urine in curative group. **A** Control; **B** lithiatic; **C** TAEE; **D** cystone

Table 2 Effect of *Triticum aestivum* ethanolic extract (TAEE) on urinary excretion parameters of lithiatic rats

Parameters (mg/dl)	Concurrent regimen				Curative regimen			
	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone
u-Creatinine	1.41 ± 0.11^{a}	9.83 ± 0.54^{b}	4.43 ± 0.06^{c}	3.03 ± 0.46^{d}	2.26 ± 0.16^{a}	11.53 ± 1.03^{b}	4.41 ± 0.77^{a}	3.81 ± 0.75^{a}
u-Urea	2.60 ± 0.14^{a}	4.01 ± 0.22^{b}	3.45 ± 0.11^{c}	2.31 ± 0.11^{a}	12.40 ± 0.47^{a}	28.31 ± 2.29^{b}	19.15 ± 0.42^{c}	12.66 ± 0.60^{a}
u-Uric acid	7.11 ± 0.08^{a}	8.61 ± 0.08^{b}	6.11 ± 0.211^{a}	7.85 ± 0.13^{c}	5.57 ± 0.12^{a}	7.41 ± 0.31^{b}	6.19 ± 0.26^{a}	5.69 ± 0.10^{a}
u-Calcium	72.41 ± 3.86^{a}	187.48 ± 10.56^{b}	105.41 ± 8.06^{c}	140.30 ± 16.04^{d}	80.16 ± 7.34^{a}	150.83 ± 20.08^{b}	110.43 ± 8.55^{a}	119.41 ± 10.79^{ab}
u-Phosphate	35.74 ± 0.07^{a}	47.70 ± 0.77^{b}	24.28 ± 1.70^{c}	38.01 ± 1.16^{a}	55.15 ± 1.20^{a}	74.85 ± 1.61^{b}	42.02 ± 2.26^{c}	46.86 ± 1.31^{c}
u-Magnesium	1.16 ± 0.03^{a}	0.84 ± 0.06^{a}	2.11 ± 0.26^{b}	2.46 ± 0.05^{b}	2.11 ± 0.13^{a}	1.29 ± 0.12^{b}	1.99 ± 0.15^{a}	2.53 ± 0.09^{c}

Values are expressed as mean ± SEM ($n = 6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P < 0.05$)

Effect of TAEE on renal calculi deposition using histological evaluation

In prophylactic and curative regimens, Figs. 3 and 4, respectively, display that control groups of both regimens showed intact renal tissue architecture (Figs. 3A, 4A). In contrast, calcium oxalate crystal deposits in intratubular were detected in the renal tissues of lithiatic rats (Figs. 3B, C, 4B, C). Histological analysis of TAEE (500 mg/kg b.wt) showed its ability to partially prevent/cure the calcium oxalate crystals deposition in renal tissue. As along the fields there is no/few crystals observed in comparison with the untreated lithiatic group in both regimens (Figs. 3D, E, 4D). Likewise, the kidney sections of lithiatic rats received cystone showed no or few crystals deposition either in prophylactic regimen (Fig. 4F, G) or in curative one (Fig. 4E). These findings suggest the efficacy of TAEE as antilithiatic agent like the standard cystone drug.

Table 3 Effect of *Triticum aestivum* ethanolic extract (TAEE) on serum kidney function markers of lithiatic rats

Parameters (mg/dl)	Concurrent regimen				Curative regimen			
	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone
s-Creatinine	17.91 ± 0.32^{a}	25.73 ± 0.26^{b}	22.61 ± 0.35^{c}	22.71 ± 0.22^{c}	25.73 ± 0.26^{a}	40.66 ± 1.88^{b}	21.68 ± 1.07^{a}	23.10 ± 2.23^{a}
s-Urea	12.16 ± 0.34^{a}	14.05 ± 0.18^{b}	11.28 ± 0.27^{a}	12.33 ± 0.54^{a}	14.93 ± 1.46^{a}	30.98 ± 3.37^{b}	13.50 ± 0.97^{a}	11.71 ± 0.36^{a}
s-Uric acid	2.96 ± 0.28^{ac}	7.10 ± 0.38^{b}	3.92 ± 0.21^{a}	3.50 ± 0.30^{c}	3.07 ± 0.18^{a}	8.16 ± 0.21^{b}	4.91 ± 0.25^{a}	5.01 ± 0.26^{a}
s-Calcium	1.25 ± 0.015^{a}	5.25 ± 0.128^{b}	2.85 ± 0.038^{a}	1.76 ± 0.025^{a}	6.56 ± 0.734^{a}	11.05 ± 0.200^{b}	5.86 ± 0.385^{a}	6.20 ± 0.107^{a}
s-Phosphate	3.08 ± 0.24^{a}	5.93 ± 0.33^{b}	4.20 ± 0.25^{c}	3.16 ± 0.24^{a}	3.86 ± 0.15^{a}	5.05 ± 0.30^{b}	4.11 ± 0.27^{a}	3.76 ± 0.17^{a}
s-Magnesium	2.73 ± 0.13^{a}	1.48 ± 0.11^{b}	2.28 ± 0.34^{a}	2.30 ± 0.13^{a}	3.11 ± 0.31^{a}	1.43 ± 0.12^{b}	1.88 ± 0.28^{ab}	2.15 ± 0.11^{bc}

Values are expressed as mean ± SEM ($n = 6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P < 0.05$)

Table 4 Effect of *Triticum aestivum* ethanolic extract (TAEE) on renal oxidative/antioxidative markers of lithiatic rats

Parameters (mg/dl)	Concurrent regimen				Curative regimen			
	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone	Control	Lithiatic	Lithiatic + TAEE	Lithiatic + Cystone
LPO (nM/g. tissue)	22.83 ± 1.31^{a}	51.21 ± 2.15^{b}	37.42 ± 1.88^{c}	26.11 ± 2.02^{a}	21.23 ± 1.83^{a}	69.48 ± 4.98^{b}	32.21 ± 2.50^{c}	20.60 ± 1.30^{a}
GSH (mg/g. tissue)	5.60 ± 0.56^{a}	3.68 ± 0.08^{b}	5.38 ± 0.65^{a}	6.86 ± 0.58^{a}	5.86 ± 1.46^{a}	1.71 ± 3.37^{b}	4.90 ± 0.97^{c}	4.23 ± 0.36^{c}
GST (U/g. tissue)	53.33 ± 2.44^{a}	21.83 ± 1.47^{b}	43.51 ± 0.44^{c}	37.73 ± 1.60^{d}	50.63 ± 3.62^{a}	25.93 ± 2.20^{b}	49.43 ± 1.20^{a}	44.25 ± 2.33^{a}
SOD (U/g. tissue)	79.16 ± 1.02^{a}	49.38 ± 1.26^{b}	84.06 ± 0.73^{c}	74.58 ± 2.66^{a}	71.08 ± 2.02^{a}	45.34 ± 6.50^{b}	79.30 ± 2.47^{a}	68.06 ± 7.97^{a}
CAT (U/g. tissue)	0.53 ± 0.017^{a}	0.21 ± 0.017^{b}	0.38 ± 0.015^{c}	0.48 ± 0.009^{d}	0.71 ± 0.04^{a}	0.29 ± 0.01^{b}	0.42 ± 0.02^{c}	0.50 ± 0.01^{c}

Values are expressed as mean ± SEM ($n = 6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P < 0.05$)

Discussion

The incidence of nephrolithiasis is growing worldwide, possibly due to the lifestyle, diet, and climate changes (Zarin et al., 2020). The accessible antilithiatic treatments in many cases are cost, ineffective and have some side effects besides their limitations in the repetition of stones (Johri et al., 2010). Thus, the preventive treatment is extremely endorsed to control the lithiasis. Phytotherapy is used in traditional medicine in kidney stones remedy (Yasir & Waqar, 2011). The present study evaluates the influence of *Triticum aestivum* ethanolic extract (TAEE) against lithiasis prompted by NaOx in male rats.

Urinary chemistry is considered the most vital factor in detecting the formed crystals. Thus, the analysis of the urinary compositions is a helpful sign of the degree of lithiasis formation related to the calculi-forming minerals (Manissorn et al., 2017). The present study showed that NaOx elicits CaOx crystals. Firstly, due to the formation of CaOx crystals depending on some urinary compositions, mostly calcium that increased in serum and urine and oxalate that is injected intraperitoneally. This is supported by the fact that oxalate has a central role in lithiasis pathogenesis and hypercalciuria is a threat aspect for the onset of renal calculi (Mandavia et al., 2013; Pandhare et al., 2021). This expectation is in line with Akila et al. (2013) and Patel and Acharya (2020), who clarified that hypercalciuria preferred the nucleation of CaOx in urine resulted in crystal growth. As lithiasis arises by reason of urine supersaturation in that case, inequality between the stone promoters and inhibitors is established. The high urinary calcium level leads to supersaturation of urinary calcium salts (Akila et al., 2013). Also, urinary pH stimulates the formation of lithiasis in various steps: including crystallization, followed by growth, and ended by aggregation and retention. As the renal calculi is pH-dependent. This considered the second reason for forming CaOx crystals in the current alkaline lithiasis group, as Manissorn et al. (2017) reported that CaOx crystals are highly promoted in alkaline urine. On the other side, decreased urinary output in lithiatic groups promote supersaturation of oxalate level; subsequently, CaOx crystals formation was initiated (Patel & Acharya, 2020). Further, the alkaline pH of the lithiatic groups potentiates the formation of CaOx crystals as low urinary pH decreases the solubility of CaOx stone in urine, and hence, urine become more saturated that initiate the crystal formation process (Patel & Acharya, 2020). In addition, the increased phosphate level in urine in the presence of oxalate provides

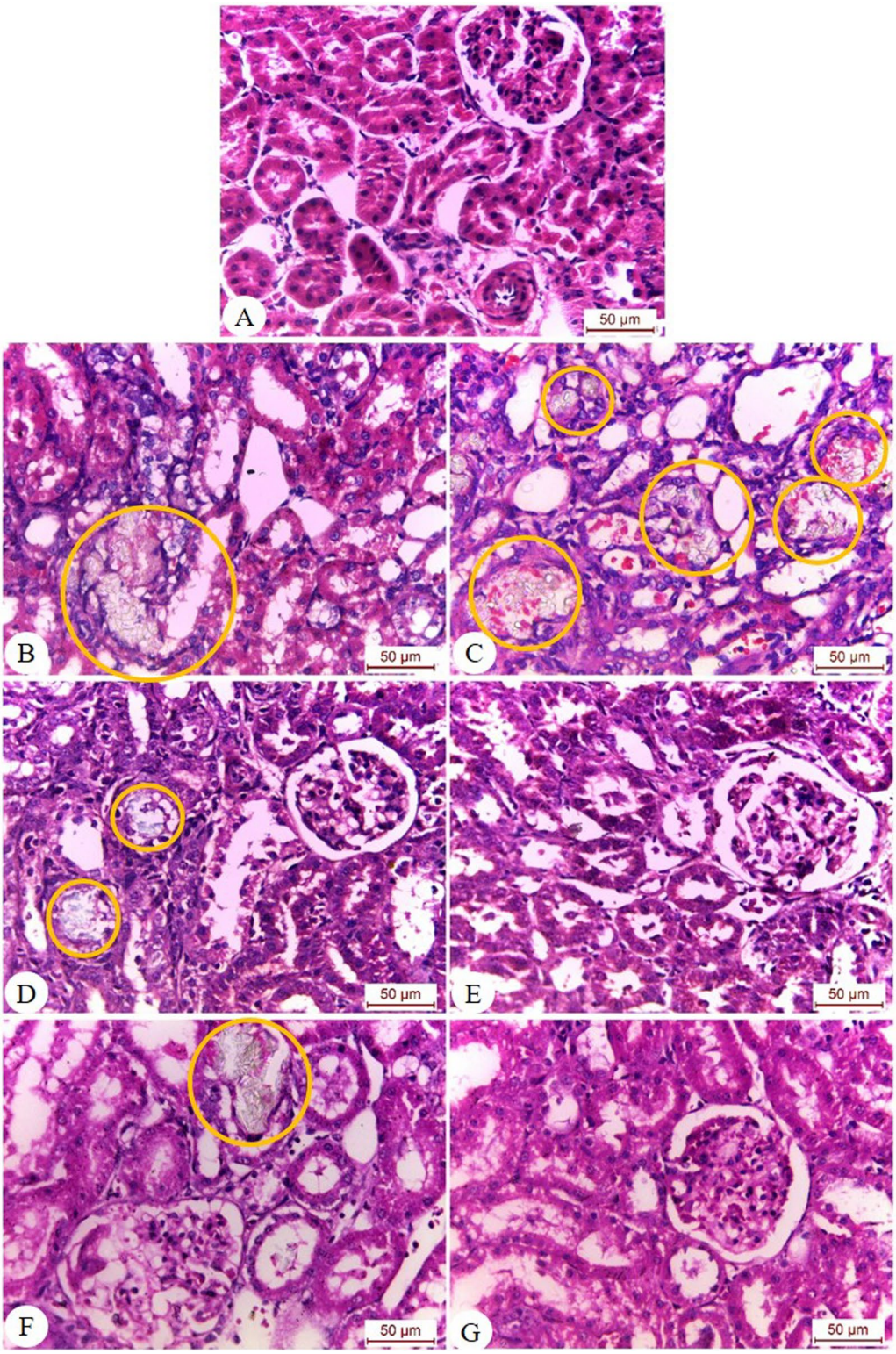


Fig. 3 Photomicrograph of kidney sections of rats in prophylactic regimen stained by hematoxylin and eosin. **A** Section of control rats showing normal architecture of glomeruli and tubular renal structure. **B**, **C** Section of lithiatic rats showing deposition of multiple crystals (yellow circle). **D**, **E** Sections of rat kidney treated with TAEE (500 mg/kg b.wt) showing marked diminution in number of crystal deposition. **F**, **G** Section of standard rats (treated with cystone 750 mg/kg) showing more or less reduction in number of crystal deposition in glomeruli and tubules

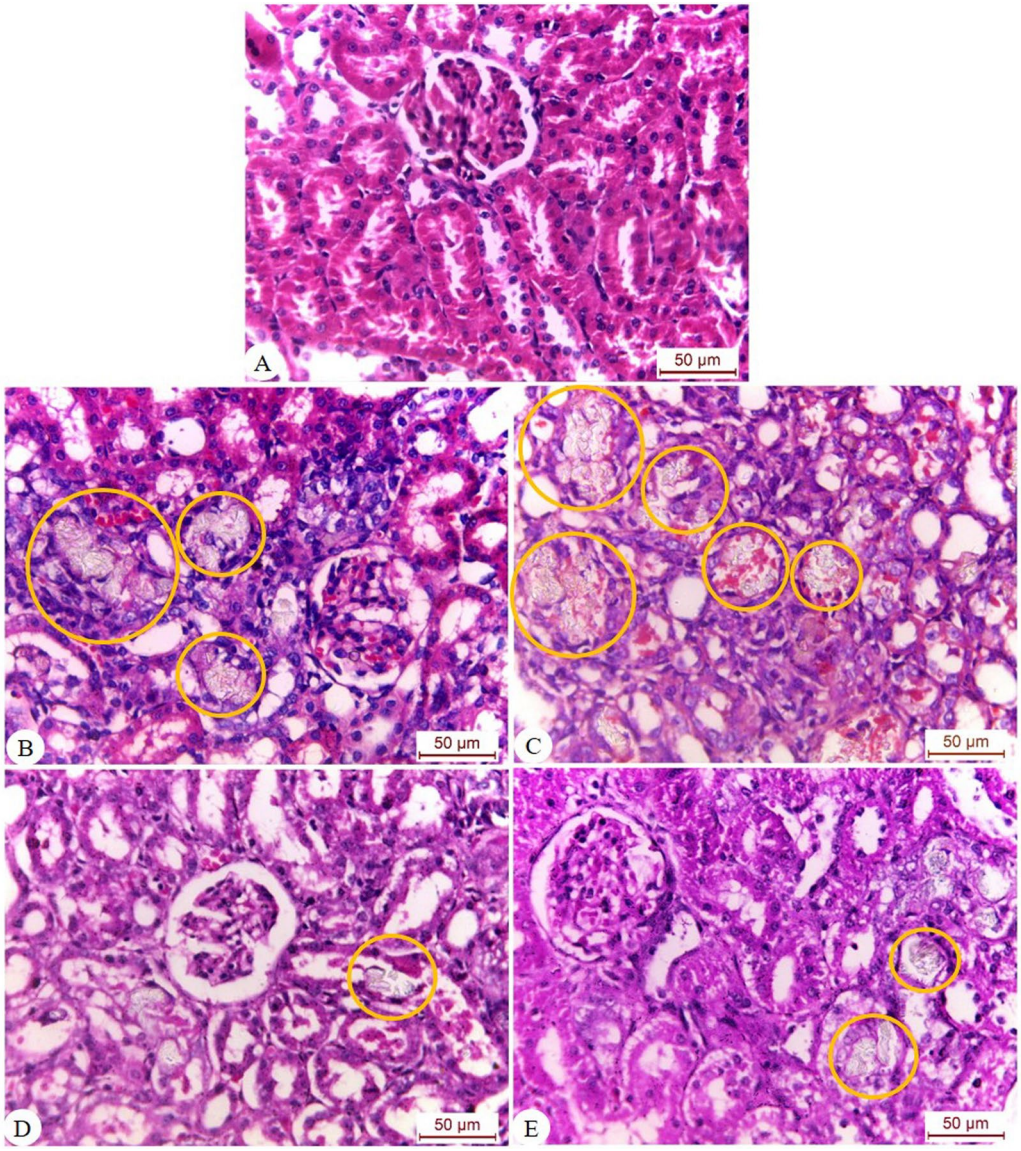


Fig. 4 Photomicrograph of kidney sections of rats in curative regimen stained by hematoxylin and eosin. **A** Section of control rats showing normal architecture of renal tissue. **B**, **C** Section of lithiatic rats showing deposition of numerous crystals (yellow circle). **D** Section of rat kidney treated with TAEE (500 mg/kg b.wt) showing marked decline in number of crystal deposition. **E** Section of rat kidney treated with standard cystone (750 mg/kg b. wt) showing significant reduction in number of crystal deposition

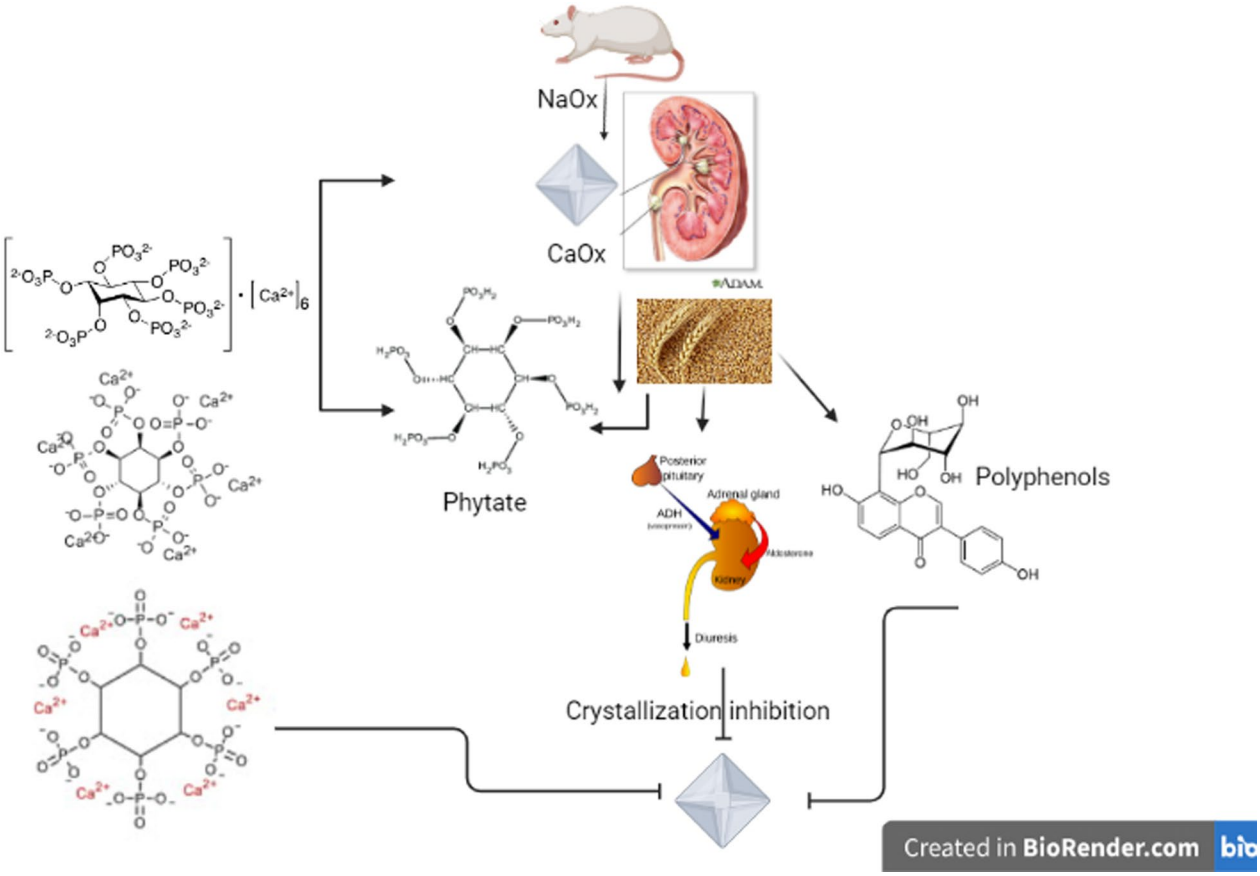
an suitable environment for formation of calcium phosphate crystals ends by stone formation (Soundararajan et al., 2006). Serum and urinary magnesium levels were reduced in the present lithiatic groups, which may supersaturate the urine and aid in the stone formation. Shah et al. (2011) stated that magnesium insufficiency hastens the deposition of CaOx crystals in renal tubules. Furthermore, the increased urinary uric acid level promotes the crystals formation (Ahmed et al., 2020). As uric acid may interfere with CaOx solubility and linked to stone inhibitors by bond, hence lessening their stone-inhibitory function (Pandhare et al., 2021). Finally, the present study assumed that lithiasis may be arisen due to the increased calcium excretion and little urinary output, which considers stone formation promoters along with lessened magnesium excretion that considered stone inhibitor. The presence of renal stones interpreted the significant elevation of serum creatinine, urea, and uric acid in lithiatic group. However, stones formation causes an obstruction to the urinary flow that origins renal dysfunction which progress to accumulate nitrogenous waste constituents in blood including creatinine, urea, and uric acid

(Ahmed et al., 2020). The above expectation about renal calculi formation is strengthened histologically by the appearance of intratubular crystals deposition in kidneys.

Oxidative stress considered as critical mediator in the lithiasis generation (Devkar et al., 2016; Sayed et al., 2020). Deposition of calcium oxalate crystal is associated with production of oxidative stress in renal tissue (Peng et al., 2015). The present study disclosed that NaOx injection initiated oxidative injury reflected by increased LPO and decreased GSH, GST, SOD, and CAT levels. The present findings are in line with the previous findings (Gupta et al., 2012; Pandhare et al., 2021; Sailaja et al., 2012; Sayed et al., 2020). The renal epithelial hurt encourages crystal retention, as epithelial damage exposes a diversity of crystal adhesion particles on epithelial surfaces (Bijarnia et al., 2008). Involvement of crystals with renal tubular tissue is a prospective factor of lithiasis (Touhami et al., 2007). This modification of oxidant/antioxidant regularity is possible owing to renal tissue impairment brought by oxalate, which reacts with PUFA (polyunsaturated fatty acids) in cellular membranes producing lipid peroxidation (Karadi et al., 2006). Additionally, Thamilselvan et al. (2000) revealed that decreased renal glutathione content prefers lipid peroxidation and maintains calcium and oxalate in the kidney.

Cystone is usually used as a gold standard medication for comparison of antilithiatic activity of plant extracts (Mekap et al., 2011; Takawale et al., 2012). So, the current study compares both regimens of TAEE with cystone to evaluate the extent of ameliorative effect of TAEE. Interestingly, TAEE exhibited a valuable effect in inhibiting stone formation and helping to treat the already formed stones. In consonance with Sekkoum et al. (2011), *Triticum aestivum* inhibit CaOx crystal development. Also, the ethanolic extract of *Hordeum vulgare* belongs to Gramineae family as *Triticum aestivum*, has preventive and curative effects on renal calculi and can be considered as antiurolithiatic (Patel & Acharya, 2020; Patel et al., 2018; Shah et al., 2012). The present study revealed significant raising in the levels of magnesium and urinary volume (stone inhibitors) in both TAEE regimens, as compared to the untreated lithiatic group. Diuretic role of a tested extract is required to rise the quantity of fluid in the kidneys and eliminate the deposits. The increased urinary output in TAEE co-/post-treated rats dilute the saturation of urinary electrolytes and confirmed the diuretic action of TAEE. This action inhibits the stone formation factors and demonstrated by noteworthy decrease of calcium and phosphates of urine. The present study attributes the diuretic effect of TAEE to its flavonoid content (Dhaliwal et al., 2015). Since flavonoids and its derivatives considered as antagonists for adenosine A1 receptor (AA1R), and hence, diuresis effect was induced (Patel & Acharya, 2020). Thus, there is a minor chance of precipitation, and infirmity for stone formation. This interprets the inhibitory role of TAEE against lithiasis formation as the antilithiatic efficacy of the natural agents returned to their diuretic role (Dodoala et al., 2009). Thus, TAEE has dual effect as it increased the stone inhibitors and diminished the stone promoters in urine. Also, administration of TAEE caused noteworthy improvement in renal functions, which proposes antilithiatic effectiveness of TAEE that prevent renal injury triggered by hyperoxaluria. Moreover, lithiatic rats treated with TAEE (prophylactic or post-treatment) significantly mitigated oxidative damage provoked by NaOx. This occurred via the drop of the renal LPO level and repletion of antioxidant molecules as compared with lithiatic rats. Thereby, the antioxidant activity of TAEE may attenuate CaOx crystal formation by reducing hyperoxaluria-induced peroxidation to the renal tubular membrane that ends by crystal attachment and renal calculi formation. The efficiency of TAEE as a preventive or curative agent in lithiasis was also established histologically. Since, the renal tissue of lithiatic rats treated with TAEE has less or none crystals relative with lithiatic untreated group. Interestingly, by comparing between TAEE and cystone as a standard antilithiatic drug, similar observations were noticed in all the above-mentioned parameters.

Finally, the present study suggests that TAEE may inhibit crystal formation via minimizing the size of crystal growth and aggregation. This suggestion is in line with Sekkoum et al. (2011), as they revealed that *Triticum aestivum* inhibit 70% crystal development and above 80% of CaOx crystal aggregation in vitro. The antilithiatic effect of TAEE may be attributed to its phytate content. Phytate is a hexaphosphoric ester of inositol that has the ability to reduce calcium absorption (Sekkoum et al., 2011), which interpreted the lower serum calcium level in the TAEE group. Sekkoum et al. (2011) explained the chelation process as the phytic acid contains 12 negative charges that can form irreversible complex with 6 mol of calcium at neutral or alkaline pH that achieved in the urinary pH of lithiasis-TAEE co-/post-treated groups. Further, the preventive and curative effects of TAEE may be due to its antioxidant potency that arising from its phytochemical constituents including phenolic compounds (Mohan et al., 2013). Kulkarni et al. (2006) reported that ethanolic extract of *Triticum aestivum* has higher phenolic content than aqueous extract. Therefore, TAEE may contain high flavonoids, alkaloids, tannins, terpenoids, saponins beside the ferulic acid that has a potency to prevent lithiasis formation (Dhaliwal et al., 2015; Zhao et al., 2019). Bawari et al. (2020) added that saponins and flavonoids have the potency to disintegrate and dissolute CaOx crystals. Also, tannins have anticrystallization action via



Scheme 2 Schematic diagram showing the antilithiatic effect of *Triticum aestivum* ethanolic extract

formation of calcium complexation and thus preventing CaOx crystal formation. All the above-mentioned properties of TAEE enable it to prevent or dissolute the renal calculi.

Conclusions

After more investigations in higher animals, the present study suggests that *Triticum aestivum* may be utilized as a dietary supplement, especially in persons prone or suffering from lithiasis. As, TAEE has promising antilithiatic activity for prevention/treatment of renal calculi probably by inhibiting calcium oxalate crystal growth (prevention) or by chelating process (treatment). These effects may be due to its polyphenolic or phytate contents, respectively (Scheme 2).

Abbreviations

CaOx Calcium oxalate
CAT Catalase
GSH Glutathione reduced
GST Glutathione-s-transferase
MDA Malondialdehyde
SOD Superoxide dismutase
TAEE *Triticum aestivum* Ethanolic extract

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Author contributions
AAS construct and carried out the scientific idea and writing the original draft.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
The protocol for conducting the acute toxicity studies and in vivo studies was performed according to the guidelines of the care and use of laboratory animals (8th edition), and the study was approved by the Institutional Animal Care and Use Committee (IACUC), (CUFS/S/PHY/49/15) of the Faculty of Science, Cairo University, Egypt.

Consent for publication
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Competing interests
There is no any potential conflict of interest.

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