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Anti-nephrolithiatic efficacy of *Teucrium polium* aerial parts extract in a lithiasic rat model

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ABSTRACT: The present study was designed to investigate the anti-nephrolithiatic potential of the aqueous extract of *Teucrium polium* in male Wistar rats. The experiment was carried out in male rats divided into 4 groups, each of which contain 6 animals. Group I (normal control) received distilled water for 40 days. Group II(nephrolithiatic control) to IV(treatment groups)animals received 1% v/v ethylene glycol (EG) in distilled water for 20 days. Group III (standard group) received Cystone® (750 mg/kg) from 20th day - 40th day. Group IV received (200mg/kg) from 20th to 40th day. Urinary volume, pH, and biochemical parameters such as calcium, uric acid, urea, phosphorus and oxalate; and serum calcium, blood urea nitrogen(BUN), uric acid, phosphorus, creatinine and magnesium were assessed. In addition, histopathological changes in kidney were carried out. Supplementation with aqueous extract of *T. polium* significantly increased urine volume (P<0.01) and urine pH (P<0.05). Aqueous extract of *T. polium* significantly reduced the elevated urinary and serum levels of uric acid (P<0.001). Moreover, it significantly lowered the urinary, serum levels of phosphorus(P<0.05) and significantly improved serum magnesium level(P<0.05). It is noteworthy to mention that *T. polium* revealed no effect on the other biochemical parameters. We can conclude that *T. polium* aqueous extract could decrease the risk of formation of CaOx stones by affecting many biochemical parameters other than oxalate.

KEYWORDS: Nephrolithiasis; kidney stone ; calcium oxalate (CaOx), *Teucrium polium* ; ethylene glycol.

1. INTRODUCTION

Nephrolithiasis or kidney stones, is an illness in which individuals form calculi within the renal pelvis and tubular lumens. The occurrence of nephrolithiasis among children and adolescents have increased hurriedly over the last 25 years. Among adults, around 50% of patients with incident nephrolithiasis will develop a recurrent stone within 5-10 years of the first kidney stones [1]. At present, no satisfactory drugs are accessible in the market for the treatment, prevention, or recurrence of kidney stone formation [2]. Synthetic drugs for the treatment of nephrolithiasis are associated with greater occurrence of adverse drug reactions. Although it is well established that invasive procedures as extracorporeal shock, wave lithotripsy, ureteroscopy and nephrolithotomy are remarkably effective for stones, there use have been linked to infections, reduced renal functions and acute renal injury. There is also a potential recurrence of kidney stones formation, in addition to the high cost [2]. Dietary and lifestyle changes that have occurred over decades promote the incidences of renal stone disease. The evidences agree on the harmful effects of high meat/animal protein intake and low-calcium diets, whereas the high content of fruits and vegetables associated with a balanced assumption of low-fat dairy products carries the lowest risk for incident kidney stone [3]. In this regard, traditional herbs have become a vital zone in the search for new drugs [4]. A regional distribution of the emphasis in nephrolithiasis research is obvious; the majority of animal studies have been performed using medicinal plants from Indian, Japanese, Brazilian, Mexican, Moroccan, Chinese and Iranian ethno-medicines [5]. More than *in vivo* anti-nephrolithiatic study has been reported for *Alisma orientale*, *Herniaria hirsuta*, *Phyllantus niruri*, *Nigella sativa*, *Trigonella foenum, Herniaria hirsute*, *Punica granatum*, *Citrus limon* and others [5].

T. polium (Lamiaceae) is a commonly used medicinal plant in folk medicine which has been used in traditional medicine as diuretic, tonic, antipyretic, anti-fungal, anti-spasmodic, anti-rheumatic, carminative and antibacterial agent. Numerous studies investigated the effects of *T. polium* against different pathological situations in different organs [6].

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In view of the traditional and ethnomedicinal use of *T.polium* for the treatment of kidney stones[7], it was thought worthwhile to investigate its effect on experimentally induced *in-vivo* lithiatic model. Hence, the present study evaluated the effect of treatment with aqueous extract of *T. polium* on laboratory rats with CaOx lithiasis induced by ethylene glycol (EG).

2. RESULTS

2.1. Acute toxicity study

No sign of toxicity was detected at the maximum administered dose (2000 mg/kg) of *T. polium*. Hence, (2000 mg/kg) dose of *T. polium* was considered as the highest tolerated dose. One tenth of the highest tolerated dose i.e. (200 mg/kg) was selected as lower therapeutic dose for or the anti-nephrolithiatic study [8].

2.2. Urine analysis

Urine volumes were increased significantly by *T. polium* ($P<0.05$) and the standard drug Cystone® ($P<0.001$) compared to the nephrolithiatic control group. *T. polium* and Cystone ® significantly increased the pH ($P<0.01$). On the other hand a significant decrease in urine volume ($P<0.01$) and pH ($P<0.05$) was observed in the animals treated with the 1% of EG (Table1).

Table 1. Effect of *T. polium* on urine volume and urine pH in nephrolithiasis induced rats.

	Normal control	Nephrolithiatic control	Cyston® (750mg/kg)	*T. polium* (200mg/kg)
Urine volume (ml/24hr)	5.42±0.37**	3.50±0.43	8.84±0.83***	5.92±0.80*
pH of Urine	6.18±0.25*	5.33±0.21	6.1±0.18**	6.58±0.27**

n=6, *$P<0.05$,**$P<0.01$,***$P<0.001$ vs. nephrolithiatic control; values are expressed in mean± SE.

Nephrolithiasis disease progression was marked by significantly raised urinary levels of calcium, uric acid and oxalate ($P<0.05$) concentration in urine of nephrolithiatic group. *T. polium* reduced urinary levels of phosphorous ($P<0.05$) and uric acid ($P<0.001$). No significant difference was observed in the values of calcium, urea and oxalate values. Cystone® significantly attenuated elevated calcium, phosphorous and oxalate($P<0.05$) (Table 2).

Table 2. Effect of *T. polium* on urine parameters in nephrolithiasis induced rats.

	Normal control	Nephrolithiatic control	Cyston® (750mg/kg)	*T. polium* (200mg/kg)
Calcium (mg/dl)	7.5±1.25*	11.83±1.86	7.1±0.76*	10.3±0.14
Phosphorous (mg/dl)	60.60±0.42	60.28±2.31	54.2±0.17*	49.15±0.24*
Urea (mg/dl)	0.30±0.01	1.67±0.34	0.4±0.22	0.17±0.14
Uric acid (mg/dl)	15.9±4.89*	20.84±0.93	17.90±1.99	10.13±0.40***
Oxalate (mmol/l)	0.65±0.02*	0.95±0.11	0.61±0.02*	0.845±0.06

n=6, *$P<0.05$,**$P<0.01$,***$P<0.001$ vs. nephrolithiatic control; values are expressed in mean± SE.

Microscopic view of urine samples from normal control group didn't show any CaOx crystals **A**. On the other hand, microscopic view of urine samples from nephrolithiatic control group depicted higher amount of CaOx crystals **B**. Microscopic view of urine samples from treated groups; Cystone® depicted fewer and small size of CaOx crystals **C**. *T. polium* treated group depicted fewer and larger size agglomerate CaOx crystals **D** (Figure 1).

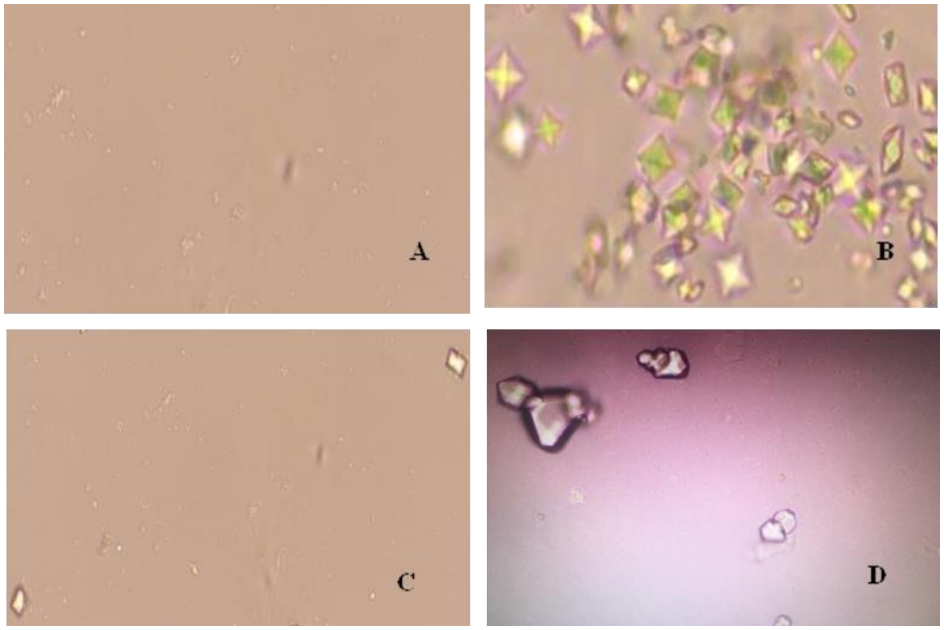

Figure 1. Representative photographs of CaOx crystals from urine samples of experimental rats as observed under light microscope (x400) in A Normal Control group, B Nephrolithiatic control group, C Cystone® treated group, D 200 mg/kg *T. polium* treated group.

2.3. Serum analysis

Significantly elevated levels of calcium (P<0.01), phosphorous(P<0.01), BUN(P<0.05), creatinine (P<0.05) and uric acid (P<0.01) were observed in serum of nephrolithiatic group *T. polium* treatment produced a significant reduction in elevated serum levels of phosphorous (P<0.05), uric acid(P<0.05) and significant increase in magnesium level(P<0.05). No significant difference was observed in the values of calcium, BUN and creatinine. Cystone® significantly attenuated elevated calcium (P<0.01), BUN(P<0.001) and uric acid(P<0.01) (Table 3).

Table 3. Effect of *T. polium* on serum parameters in nephrolithiasis induced rats.

	Normal control	Nephrolithiatic control	Cyston® (750mg/kg)	*T. polium* (200mg/kg)
Calcium (mg/dl)	9.77±0.93**	10.82±0.13	10.08±0.14**	11.17±0.12
Phosphorous (mg/dl)	6.08± 0.21**	7.9 ± 1.19	8.14±0.18	6.35±0.19 *
BUN (mg/dl)	66 ±0.93*	81.17±4.3	50.4±1.33***	72.67±2.86
Uric acid (mg/dl)	2.7 ±0. 0.18**	3.75±0.13	2.02±0.09**	3.37±0.11*
Creatinine (mg/dl)	0.5±0.03*	0.59±0.02	0.55±0.01	0.55±0.03
Magnesium (mg/dl)	1.93±0.08	2.25±0.38	2.54±0.09	2.85±0.09*

n=6, *P<0.05,**P<0.01,***P<0.001 vs. nephrolithiatic control; values are expressed in mean± SE.

Furthermore, histopathological interpretations of renal tissue from nephrolithiatic revealed marked degenerative changes, necrosis and notable intratubular crystal deposition compared with normal control. Cystone® treated groups showed near normal histology. While, *T.polium* treatment group revealed necrosis and less crystal deposition (Figure2) .

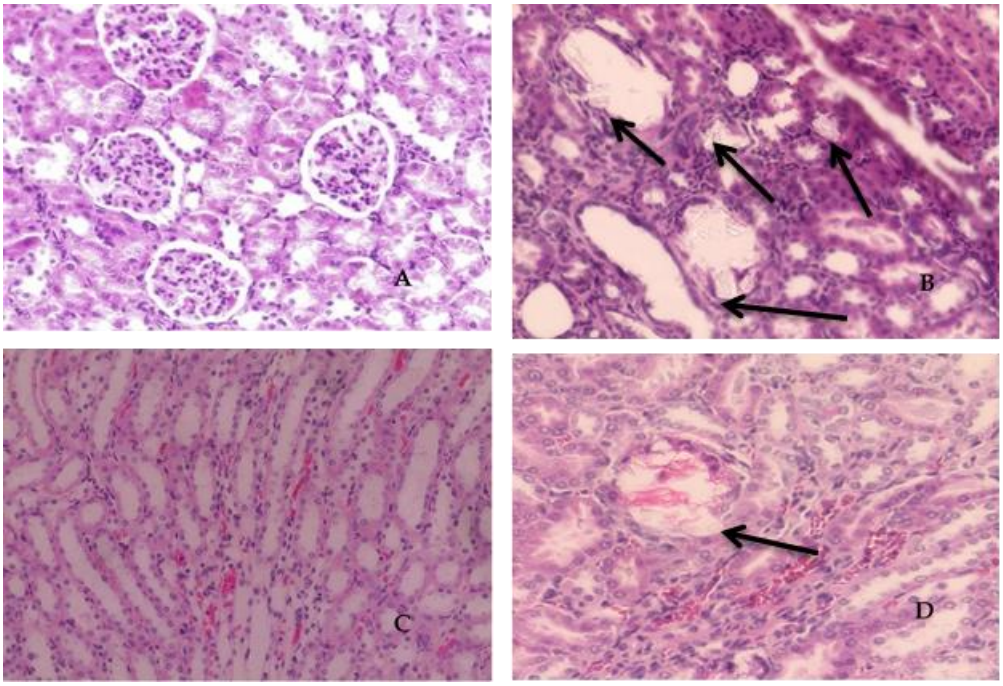

Figure 2. Representative images of Haematoxylin and Eosin stained kidney sections (x400) from **A** Normal Control group, **B** Nephrolithiatic control group, **C** Cystone treated group, **D** 200 mg/kg *T. polium* treated group. Black arrows indicate CaOx crystal depositions.

3. DISCUSSION

CaOx nephrolithiasis is the most predominant type of kidney stones compared to other types [9]. In the present study, male rats were used in order to induce nephrolithiasis, because the urinary system of male rats is similar to that of humans. Previous studies have also shown that the amount of stone deposition in female rats was meaningfully less than that of male rats[10].EG induced hyperoxaluria model of nephrolithiasis was selected and the fact that it induces hyperoxaluria which is a critical risk factor for CaOx nephrolithiasis in clinical setting.The biochemical mechanisms for these progressions indicate that the oral administration of EG (precursor of oxalate), prompts oxalate lithiasis in rats by being converted to endogenous oxalic acid in the liver [11].Cystone® was used as a standard drug in the current study considering its polyherbal as well as its popularity nephrolithiasis treatment in clinical practice [12].The dose of Cystone® was selected depend on previous studies.

Since EG is reported to be nephrotoxic, its effect on renal function was evaluated by measuring urine volume and pH, in addition to urinary levels of calcium, uric acid, phosphorus and oxalate. Furthermore, serum calcium, BUN, uric acid, phosphorus, creatinine and magnesium. On 40th day of the experiment, a significant decrease in urine volume in lithiatic group indicates formation of CaOx crystals lead to urinary tract obstruction to the outflow of urine[13].Diuretic action is necessary to increase the quantity of fluid going through the kidneys and flush out the deposits. In the existing study, urine volume was significantly increased by the aqueous extract of *T.polium* when compared to the disease control. This result supports the previous research which linked the diuretic effect with *T.polium* and demonstrated that the presence of flavonoids, glycosides, saponins, carbohydrates, tannins, terpenoids, that may encourage the proposition that these types of polar compounds may be responsible for the diuretic effect [14].In the present study, the decrease in urine pH from 6.18 - 5.33, supports the formation of CaOx type of stones. In the aqueous extract of *T.polium* administered rats, restored urinary pH [15].

Increased urinary calcium is a key factor,favours the nucleation and precipitation of CaOx from urineand consequent crystal growth. Increased urinary phosphorus excretion and oxalate stress appears to provide an environment for the formation of calcium phosphate crystals, which epitaxially increased CaOx deposition[16].Urinary obstruction due to large stones results in reduced glomerular filtration and subsequent accumulation of nitrogenous wastes such as creatinine, BUN and uric acid in blood[17].This was also evident in the present study.

On treatment with the aqueous extract of *T.polium* medicinal plant, a significant decrease in the urinary uric acid was observed. This result seem to be consistent with other research which found that *T.polium* has inhibitors effect on xanthine oxidase activity. Phenol, anthocyanin and soluble sugar content of *T.polium* may responsible for this inhibitory effect [17].That raises the possibility of effectiveness of *T.polium* against other types of kidney stones like uric acid stone. Since the main cause of uric acid stones formation is the levels of uric acid in the urine is too high[18].Treatment with extracts of *T.polium* lowered the excretion of phosphorus and restored serum phosphorus levels.

Urinary magnesium was diminished in kidney stone. Since it is an inhibitor, complexes with oxalate and decreases the supersaturation of urine[19].

Diets rich in magnesium have found to protect the kidney from deposition of CaOx [20].Treatment with extracts of *T.polium* restored serum magnesium levels. However, it did not reduce the super-saturation of calculogenic ions such as calcium and oxalate in urine. These results are likely to be related to the administered dose of *T.polium* treatment or to the size of forming stones which might be too large to be fully break up with *T.polium* treatment. The histopathological study of kidney showed degenerative changes, necrosis and notable intratubular crystal deposition in nephrolithiatic control group.Wherea,*T.polium* treatment showed less changes and crystal deposition in comaprision with nephrolithiatic control group. These findings showed the effectiveness of *T.polium* extract when compared with nephrolithiatic control group.

It is reported that Cystone® (an Ayurvedic formulation) is useful in the management of nephroithiasis, which modifies the crystalloid-colloid balance and disintegrates the stones. Cystone® acts on mucin, which binds the particles together in a calculus. By the asset of diuretic action,Cystone® flushes+ the urinary passage[11].Furthermore, it relaxes the smooth muscule of the urinary tract, thus relieves the spasms.The effect of *T.polium* extract was found to be more or less comparable to Cystone®.Therefore, it can be concluded that the *T.polium* may exert its effect through similar mechanisms.

4. CONCLUSION

Current study for the first time provided scientific credence to the folklore claiming anti-nephrolithiatic potential of *T.polium*. The presented data show that administration of the (200 mg/kg) aqueous *T.polium* extract of aerial parts to rats with EG induced lithiasis, possessed significant diuretic effect, pH changes, restored the diminished urinary and serum of uric acid and phosphorus levels and enhanced serum level of magnesium. That could decrease the risk of formation of CaOx stones. However, further studies are needed to clarify the exact mechanism of action of *T.polium* in decreasing risk of kidney stone formation.

5. MATERIALS AND METHODS

5.1. Chemical and apparatus

Ethylene glycol was obtained from Merck, Germany. The standard drug herbal formulation Cystone® was bought from Himalaya, India. Apparatus such as the metabolic cage was obtained from Teeniplast, Italy and diagnostic kits were obtained Jenway, UK.

5.2. Plant material

Arial parts of *T. polium* were purchased from traditional herbal market-Amman, Jordan. The taxonomic identity of the plant was confirmed by comparing with those of known identity which are located in the Herbarium of the Dept. of Biological Science, Faculty of Science, University of Jordan with the help of Prof. Sawsan Oran Dept. of Biological Science, Faculty of Science, University of Jordan. A voucher specimen No. TAC 2019 was deposited at the Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan (Phytochemistry lab).

5.3. Preparation of plant extract

We selected for the use of maceration and decoction in order to get as close as possible to the most traditionally use methods. Thirty grams of finely ground plant was soaked in 500ml of distilled water, mixed and left to stand 24h with continues shaking. From time to time, mixture was shaken. Plant material was then boiled for 15 min at 100°C Then, it was filtered and lyophilized (1.87g). The extract was subjected to toxicological and anti-nephrolithiasis tests.

5.4. Experimental animals

Male Wistar laboratory rats (weight range 170–200 g) were housed in polypropylene cages and maintained at 12 h light/dark cycle under controlled temperature (22-25°C) and relative humidity (50-60%). They were allowed free access to standard pellet food and water ad libitum. Animal care and use were conducted according to standard ethical guidelines, and all of the experimental protocols were approved by the Research and Ethical Committee at the Faculty of Pharmacy-Applied Science University, Amman, Jordan (Approval code: Pharm-8-2019).

5.5. Acute toxicity study

Acute toxicity study of *T. polium* was performed using acute toxic class method of acute oral toxicity determination proposed in Organization for Economic Co-operation and Development (OECD)423 guidelines [21]. As the safety profile of *T. polium* was previously reported and limit test was performed [8]. The highest dose (2000 mg/kg.) was tested on innulliparous and non-pregnant female Wistar rat (n = 6). Dosed rats were kept under observation for clinical signs of toxicity for duration of 14 days. Highest accepted dose of *T. polium* was then determined based on Globally Harmonised Classification System for Chemical Substances and Mixtures (GHS) classification [21].

5.6. Experimental design

Rats were divided into four groups (n = 6) and EG (1%v/v in drinking water) was administered to group II–IV for 20 days [22].

- Group I served as normal control and received drinking water ad libitum.
- Group II served as nephrolithiatic control and received EG in drinking water ad libitum.
- Group III served as co-treated with standard drug, Cystone® (750 mg/kg 20th day - 40th day).
- Group IV served as co-treated with *T. polium* (200mg/kg20th day - 40th day).

Distilled water was used as vehicle for suspending Cystone® and *T. polium* dried powder for the preparation of doses and were administered in experimental rats by oral gavage. The doses of Cystone® (750 mg/kg) and *T. polium* (200mg/kg) were selected according previous studies [2] [13] [23].

5.7. Urine analysis

After 40 days experimental period, rats were individually placed in metabolic cages. Their 24 h urine samples were collected. Volume and pH of urine samples was determined followed by dipstick urine analysis prior to centrifugation at 2500 rpm for 5 min. Urine samples were then subjected to microscopic evaluation of crystalluria and quantitative assessment of urea, calcium, phosphorus, uric acid on Jenway Genova plus spectrophotometer using respective diagnostic kit. Oxalate content in urine was determined by enzymatic colorimetric method [23].

5.8. Serum analysis

Following urine collection, blood was collected from the retro-orbital plexus of the rats under mild anesthesia. Serum was separated from the blood by centrifugation at 3000 rpm for 15 min. The serum thus obtained was then subjected to the quantitative assessment of BUN, uric acid, creatinine, calcium, magnesium and phosphorous using respective diagnostic kits on Jenway Genova plus spectrophotometer.

5.9. Histopathological analysis

The left kidney was fixed in 10% solution of neutral buffered formalin (pH 7.4). After processing, the tissue was embedded in paraffin and the sections of 5 µm were cut using microtome and stained with hematoxylin–eosin for assessment under optical microscope.

5.10. Statistic evaluation

Data were analyzed with SPSS software (Version 16.0, SPSS Inc, Chicago, IL) using one-way ANOVA followed by *Dunnett's* multiple comparison test using 5% level of significance.

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