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Prophylactic and curative potential of peppermint oil against calcium oxalate kidney stones

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Abstract: *Mentha piperita* L., a well-known traditional herb, constitutes essential oil as one of its important constituent, used for its flavor, aroma and therapeutic applications. Based on the antioxidant, antispasmodic and nephroprotective potential, the essential oil of *Mentha piperita* was evaluated for its preventive and curative effects against ethylene glycol induced urolithiasis. Peppermint oil (Mp.Eo) was evaluated for its antioxidant potential by DPPH method. Urolithiasis was developed in male rats by the administration of ammonium chloride and ethylene glycol in drinking water. Different doses of Mp.Eo (10, 30 and 50 mg/kg) and cystone, the standard antiurolithic drug (500 mg/kg), were given along with stone-inducing regimen in prophylactic model and after intoxication for the next fourteen days in curative model. Urine and serum were analyzed for various biochemical parameters. One representative kidney from each group was studied for changes in histological parameters. Mp.Eo was found to be effective against urolithiasis-associated changes including crystalluria, polyuria and acidic urine. Mp.Eo also neutralized the altered levels of urinary uric acid, magnesium, total protein, serum creatinine and serum BUN. The data obtained from the present study demonstrated the therapeutic importance of peppermint oil against urolithiasis.

Keywords: Curative, peppermint oil, prophylactic, urolithiasis.

INTRODUCTION

Urolithiasis is the third most prevalent disorder of the urinary system, characterized by the presence of calculi in the urinary tract including kidney, bladder or ureter. It is one of the oldest diseases affecting mankind as evident from the occurrence of kidney stones in the egyptian mummies that dates back to 4000 BC. The estimated recurrence rate of urolithiasis without preventive measures is approximately 10% in 1 year, 33% in 5 years and 50% in 10 years (Doddametikurke *et al.*, 2007). Calcific stones account for more than 80% that are composed of calcium oxalate (monohydrate and dihydrate forms) alone or in combination with calcium phosphate (Tiselius, 2005). Urolithiasis is a multifaceted process involving urinary super-saturation, crystal nucleation, aggregation, growth and retention in renal tubular epithelium. Hyperoxaluria and crystal binding to renal epithelial membrane can act in several ways like altering membrane surface to enhance crystal attachment, disrupting cellular membrane integrity, promoting mitochondrial damage and increasing the production of free radicals which finally cause renal dysfunction. Renal epithelial damage is a predisposing factor in crystallization and the product of cellular injury acts as heterogeneous nucleus in crystal aggregation and growth (Bashir and Gilani, 2009). Despite of the substantial development in allopathic medicine and progress in physical and biological manifestations of kidney stones, there is no satisfactory pharmacological treatment available in clinical practice. Extracorporeal shock wave lithotripsy and endoscopic stone removal have modernized the treatment strategies of urolithiasis but do not prevent its recurrence. Various drug treatments including alkali-citrate and thiazide diuretics have also shown some likelihood to prevent renal calculi, yet the scientific data for their effectiveness is less convincing (Hess, 2003).

According to the World Health Organization, the therapeutic use of herbs and/or plants and their derivatives is increasing as they provide an economical and affordable source of drugs for seventy-five percent of the world population (Khan *et al.*, 2021). Genus *Mentha* is known for its economically and medicinally useful aromatic species with important bio-prospects. One of the common species of *Mentha* is *Mentha piperita*, which is mainly cultivated for the production of essential oil and/or utilized as medicinal and flavoring agent in many countries (Nickavar *et al.*, 2008). Peppermint oil is a volatile secondary metabolite of *Mentha piperita* L. with a strong menthol rich fragrance. It is characterized by the presence of oxygenated monoterpenes such as menthol, menthone, 1,8-cineole, menthofurone, sabinene hydrate and menthyl acetate (Abdellatief *et al.*, 2017). Peppermint oil has been reported to possess antimicrobial, antioxidant (Singh *et al.*, 2015), antiviral (Schuhmacher *et al.*, 2003), anti-inflammatory (Juergens *et al.*, 1998), antispasmodic potential (Heghes *et al.*, 2019) and effective against hepatotoxicity, nephrotoxicity (Bellassoued *et al.*, 2018), liver fibrosis (Ogaly *et al.*, 2018) and diabetes (Abdellatief *et al.*, 2017). The aim of present study was to evaluate the preventive and curative effects of peppermint essential oil against urolithiasis. For this purpose calcium oxalate urolithiasis was induced in male albino rats and various biochemical parameters (such as urinary uric acid,

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urinary magnesium, urinary calcium, phosphate, urinary total protein, serum creatinine and serum BUN levels) and histological slides of kidney section were evaluated. The antioxidant potential was also determined as it a powerful tool in preventing calcium oxalate crystal-induced cellular injury.

MATERIALS AND METHODS

Procurement of peppermint essential oil

Essential oil of pure therapeutic grade (Mp.Eo; *Mentha piperita*) was procured from an ISO certified company, Co NATURAL Pvt Ltd, Lahore, Pakistan with batch number; PEP-004458.

Determination of antioxidant potential

Antioxidant activity of Mp.Eo was evaluated by using 0.1 mM of DPPH solution. Different dilutions of ascorbic acid and Mp.Eo were prepared in methanol (5-150µl/ml) and DPPH solution (1 ml) was added to each dilution (3 ml). Solutions were allowed to stand at room temperature for 30 min and absorbance was measured at 517nm (Bashir and Gilani, 2009; Javed *et al.*, 2020). Antioxidant potential was calculated by following equation:

Percent DPPH radical-scavenging = $[1-(A_A-A_B)/A_A]\times100$

(A_A=Absorbance of control and A_B=Absorbance of sample)

Animal Model of Urolithiasis

All the experimental procedures were performed in compliance with the rules of Pharmacy Animal Ethics Committee (PAEC) of the Islamia University of Bahawalpur, under reference number; PAEC/2020/27. Wistar Albino rats (male), weighing 180–220 g, were kept in the polycarbonate cages with sawdust (renewed after every 48 h), under controlled temperature of 23±2 °C and 12 h light/dark cycle. The rats were divided in different groups, each comprising of 4 rats. Uroliths were induced in rat's kidney by giving 0.75% ethylene glycol (EG) (Merck, Germany) for 21 days and 1% ammonium chloride (AC) (Merck, Germany) for first 5 days only, in tap water (Bashir and Gilani, 2011). Normal control and intoxicated groups received 1% tween 80 (5 ml/kg, p.o once daily). Treatment groups received different doses of Mp.Eo; i.e. 10, 30, 50mg/kg along with lithogenic regimen in prophylactic study model and after intoxication for the next 14 days in curative study model. Cystone (Himalaya, India. and Batch no. 112000673), consist of extracts of *Veronoia Cinerea, Didymocarpus pedicellata, Saxifraga ligulata, Cyperus scariosus, Rubia cordifolia, Achyranthes aspera, Onosma bracteatu* and powder form of *Hajrul yahood Bhasma* and *Shilajit*, at the dose of 500 mg/kg, was used as standard drug (Das and Malipeddi, 2016).

Biochemical analysis

Urine Analysis

Fresh morning urine was examined for the presence of crystals and 24h urine was collected for further biochemical analysis; i.e. urine volume, urine pH, uric acid, magnesium, calcium, phosphate and total protein levels.

Serum Analysis

At the end of 21st day (prophylactic urolithiasis model) and at the end of 21st and 35th day (curative urolithiasis model), the rats were anesthetized (by using ketamine and xylazine) and blood was collected through retro-orbital puncture.

Serum was separated by centrifugation of blood at 4000 rpm for 10 min and analyzed for creatinine and urea nitrogen levels by using commercially available kits (Human Diagnostics Worldwide, Germany).

Histological analysis

At the end of the urolithiatic model, animals were dissected. One representative kidney from each group was preserved in 10% formalin (Riedel-de Haen, Germany) solution for histological investigation. Tissues were sectioned and stained with haematoxylin and eosin dyes, to examine morphological changes under light microscope (100X).

Acute toxicity assay

Twenty-five mice of either sex were divided into different groups of five mice each. The animals were fasted overnight. Control goup was given 1% Tween 80 (10 ml/kg, p.o). Treatment groups were administered with different doses (0.5, 0.1, 5, 1g/kg, p.o) of Mp.Eo, followed by observation for the effects; i.e. behavior pattern (alertness, grooming, touch response, pain response and convulsions), urination, sweating, writhing reflex and lacrimation for 24 hours (6 hourly), 48 hours and then regularly for 14 days (Rasheed *et al*., 2016).

STATISTICAL ANALYSIS

The results were analyzed using one-way ANOVA and the values were expressed as mean ± SEM. Graph Pad prism 8 was used for statistical interpretation of data; i.e. the values are considered non-significant if $p>0.05$, significant if $^{*}p<0.05$, more significant if $^{**}p<0.01$ and highly significant if $^{***}p<0.001$.

RESULTS

Antioxidant potential of Mp.Eo

The essential oil of *Mentha piperita* (Mp.Eo) neutralized DPPH and maximum inhibition of 78.34% was observed at the concentration of 150µg/ml with IC_{50}=42.27µg/ml.

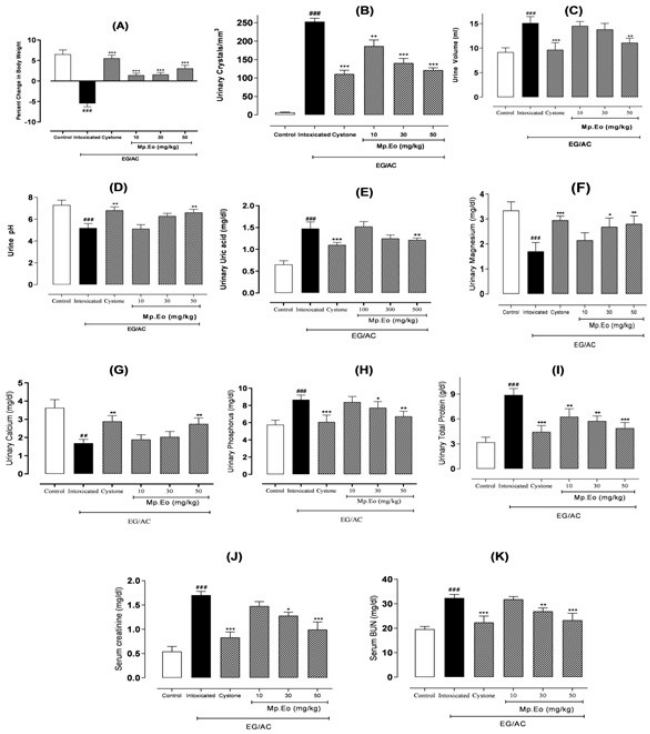

Fig. 1: The graphs showing effects on (A) percent change in body weight, (B) urinary crystal count, (C) urine volume/100g/24hr, (D) urine pH, (E) urinary uric acid (F) urinary magnesium (G) urinary calcium (H) urinary phosphate (I) urinary total protein, (J) serum creatinine and (K) serum BUN levels after consumption of AC/EG, along with the different doses of Mp.Eo and Cystone on 21st day in prophylactic model of urolithiasis. (Values are expressed as: mean ± SEM, Levels of significance: *P<0.05, **P<0.01, ***P<0.001 vs. intoxicated; $^{\#\#\#}$P<0.05 vs. control).

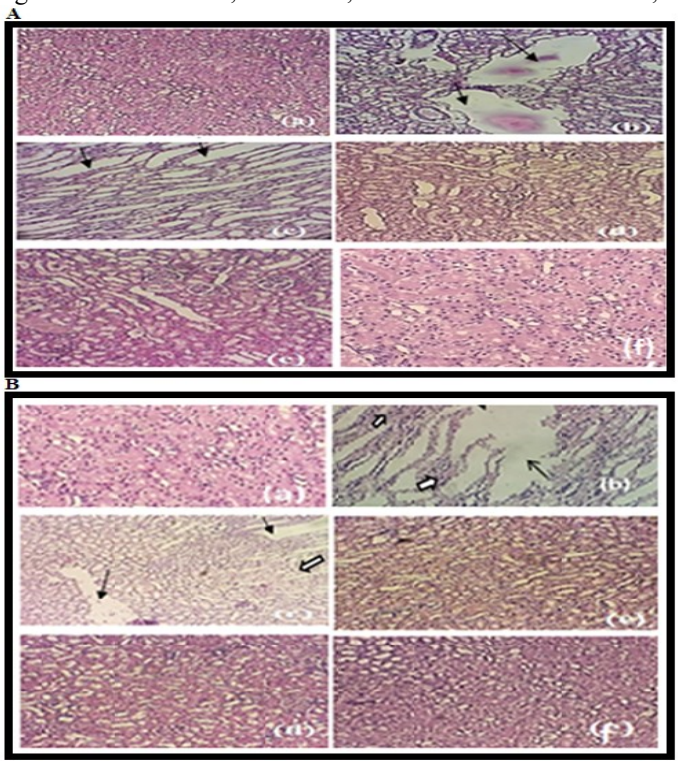

Fig. 2: The histological slides of kidney section; (a) Control, (b) Intoxicated, (c) Mp.Eo (10mg/kg), (d) Mp.Eo (30 mg/kg), (e) Mp.Eo (50mg/kg) and (f) Cystone (500 mg/kg) ; (A) prophylactic and (B) curative urolithiasis model, arrow showing deformities (⇨) and enlarged interstitial spaces (→).

Table 1: The effects of peppermint essential oil (Mp.Eo) on various parameters in albino rats using EG/AC-induced curative urolithiasis model

		Day	Control	Intoxicated	Cystone (500 mg/kg)	Mp.Eo		
						10 mg/kg	30 mg/kg	50 mg/kg
Percent change in body weight		21^{st}	5.44±0.97	-6.62±1.19	-8.08±0.53	-7.45±0.66	-7.82±0.71	-9.74±1.12
		35^{th}	5.52±1.01	1.19±0.33	5.33±0.63***	2.33±0.43***	2.59±0.39***	5.28±0.86***
Urinary parameters	Crystal count (/mm^3)	21^{st}	4.5±1.3	236±6.84	221.3±10.56	228.3±9.93	232.8±5.36	229±8.51
		35^{th}	5±0.91	234.5±7.94	146.8±14.41***	217.3±8.84*	188.5±10.4***	168.5±18.96***
	Volume (ml)	21^{st}	9.75±1.47	15.93±1.32	15.33±1.28	17.18±1.52	16.93±1.7	14.9±1.09
		35^{th}	10.2±1.13	15.35±1.82	11.55±1.34***	15.05±1.85*	13±1.62**	11.38±0.88***
	pH	21^{st}	7.7±0.31	5.98±0.28	5.01±0.35	5.08±0.37	5.76±0.44	5.37±0.19
		35^{th}	7.58±0.3	5.51±0.21	6.94±0.25***	5.2±0.32	6.51±0.28**	6.68±0.33**
	Uric acid (mg/dl)	21^{st}	0.66±0.05	1.31±0.14	1.52±0.11	1.37±0.06	1.5±0.13	1.56±0.08
		35^{th}	0.67±0.06	1.45±0.12	0.96±0.1***	1.29±0.02^{ns}	1.27±0.07*	1.24±0.04**
	Magnesium (mg/dl)	21^{st}	3.66±0.44	1.99±0.29	1.75±0.21	1.36±0.11	1.59±0.31	1.57±0.2
		35^{th}	3.65±0.55	1.88±0.27	3.05±0.3***	2.14±0.12*	2.49±0.14*	2.95±0.29**
	Calcium (mg/dl)	21^{st}	3.92±0.25	1.86±0.23	1.77±0.24	1.71±0.19	1.57±0.2	2.03±0.28
		35^{th}	4.12±0.23	1.79±0.19	2.32±0.33***	1.81±0.2	1.66±0.19	2.36±0.16***
	Phosphate (mg/dl)	21^{st}	5.87±0.33	8.55±0.39	9.03±0.47	8.29±0.55	8.93±0.54	8.86±0.38
		35^{th}	5.85±0.31	8.57±0.46	6.02±0.81***	7.57±0.36	6.81±0.31*	6.24±0.32**
	Total protein (g/dl)	21^{st}	3.72±0.87	8.75±0.55	8.58±0.98	8.64±0.64	8.66±0.37	9.27±0.59
		35^{th}	3.79±0.73	8.33±0.61	5.09±0.71***	6.97±0.57	5.94±0.7**	5.25±0.56***
Serum parameters	Creatinine (mg/dl)	21^{st}	0.61±0.06	1.59±0.12	1.51±0.16	1.41±0.06	1.28±0.1	1.3±0.11
		35^{th}	0.55±0.08	1.67±0.01	0.77±0.1***	1.21±0.04	1.05±0.13*	0.91±0.09**
	BUN (mg/dl)	21^{st}	19.12±2.02	34.21±2.02	33.30±2.39	33.11±0.82	34.54±1.58	32.80±1.2
		35^{th}	17.97±1.98	33.19±2.04	23.1±1.89***	30.24±1.2	26.98±1.62*	23.35±0.7**

(Values are expressed as: mean ± SEM, Levels of significance: *P<0.05, **P<0.01, ***P<0.001 vs. 21^{st} day of respective group.)

Acute toxicity assay

Mp.Eo was found safe up to the dose of 1 g/kg as there were no signs of toxicity observed till 14 days.

Prophylactic and curative models of urolithiasis

Percent Change in Body Weight

Lithogenic group showed significant reduction (p<0.001) in percent change in body weight after intoxication as compared to the normal control group. Significant increase in percent change in body weight was observed, at all the doses of Mp.Eo (10, 30 and 50mg/kg) as well as cystone, shown in fig. 1A (for prophylactic model) and table 1 (for curative model).

Urinary Parameters

In prophylactic model, lithogenic group produced greater number of urinary crystals (253±9.34/mm^3) as compared to the control group (6.25±1.25/mm^3). Mp.Eo produced significant dose-dependent effects by reducing the number of crystals; i.e. 186.8±16.57/mm^3, 140.5±12.61/ mm^3 and 121.5±6.27/mm^3, at the doses of 10, 30 and 50 mg/kg (fig. 1 B). In curative study model, various doses of Mp.Eo and cystone at 35th day (after 14 days of treatment) also showed significant reduction in crystal count as summarized in table 1. In both the models, urine volume was observed to be increased in lithogenic group as compared to control group. Mp.Eo, at the dose of 50 mg/kg, reduced the urine volume significantly (fig. 1C and table 1). In diseased control group (prophylactic model), pH of urine decreased up to 5.19±0.4 as compared to control group (7.29±0.45) (fig. 1D). Both in prophylactic and curative models, treatment with Mp.Eo neutralized the acidic pH in dose-dependent manner (fig. 1D and table 1). Mp.Eo, at all the doses, showed reduction in urinary uric acid levels in both the models as compared to intoxicated group (fig. 1E and table 1). In both (prophylactic and curative) models, decrease in urinary magnesium levels was observed in intoxicated group which was significantly increased in standard (cystone) and Mp.Eo treated groups (fig. 1F and table 1). Decrease in calcium concentration was observed in intoxicated group and Mp.Eo, at 50mg/kg, improved urinary calcium levels (fig. 1G and table 1). In prophylactic and curative models, phosphate and total protein levels were abnormally increased in lithogenic group and the treatment with cystone and Mp.Eo showed significant reduction in the levels (fig. 1H, 1I and table 1).

Serum Parameters

In both the models, serum creatinine and serum blood urea nitrogen levels in lithogenic group were highly significantly (p<0.001) increased as compared to the control group. Mp.Eo and cystone neutralized creatinine and BUN levels in dose-dependent fashion (figs. 1J, 1K and table 1).

Histological examination

Intoxication with EG, caused the structural disorganization with enlarged interstitial spaces as compared to the normal control group as revealed from

histological examination of kidney section. Treatment with different doses of Mp.Eo (10, 30 and 50mg/kg) improved the renal tubular integrity significantly and improved the interstitial spaces between the cells. Mp.Eo at the dose of 50mg/kg was found to be effective against EG-induced renal epithelial injury, as shown in fig. 2A for prophylactic model and 2B for curative model.

DISCUSSION

Urinary super saturation along with stone-forming constituents is generally considered one of the causative factors in lithogensis. Administration of ammonium chloride (AC) and ethylene glycol (EG) to the rats resulted in the formation of urinary crystals mainly composed of calcium oxalate. Male Albino rats were used in the study, as circulating testosterone is directly linked with hepatic glycolate oxidase and increased serum testosterone concentration caused increase in oxalate production by liver (Khan, 1997). Ammonium chloride has been reported to accelerate the stone formation as it causes urinary acidification (Atmani *et al.*, 2003). In prophylactic and curative models, percent change in body weight was decreased in intoxicated group which was significantly increased when the animals were treated with different doses of Mp.Eo. The improvement in body weight with different plant extracts; e.g. *Bergenia ligulata, Berberis vulgaris* and *Desmodium styracifolium,* etc have been reported in earlier studies (Bashir and Gilani, 2009; Bashir *et al.*, 2010; Zhou *et al.*, 2018). Microscopic examination of fresh morning urine showed greater number of calcium oxalate crystals in the intoxicated group as compared to those in control group. Mp.Eo caused decrease in crystal count as well as reduced the colic, as peppermint oil is reported for its spasmolytic potential due to the interference of menthol with the movement of Ca^{++} across the cell membrane (Heghes *et al.*, 2019).

Calcium oxalate crystals exist in two polymorphs, the monohydrate form, which is more likely to get attached with renal epithelium and aggregates easily as compared to the dihydrate form. Treatment with Mp.Eo converted monohydrate crystals into dihydrate form that were smaller in size so that these crystals can easily pass in urine, without damaging renal tubules. Urinary output was significantly increased in intoxicated group as compared to normal control which could be due to the renal epithelial damage that causes decreased water reabsorption, as already reported (Divakar *et al.*, 2010). Treatment with Mp.Eo prevented polyuria associated with lithogenic treatment, although urine output remained higher than that of the control group which may be due to the diuretic potential of peppermint, as reported traditionally (Ahmed *et al.*, 2017). The present study showed that Mp.Eo improved urinary pH, that affects the solubility of crystals and prevented crystal nucleation, precipitation and aggregation as already discussed in previous studies (Abhirama and Shanmuga, 2018). Increased levels of uric acid were observed in urolithic rats. Uric acid disturbs the inhibitory action of glycosaminoglycan (mucopolysaccharide) by forming gel phase in urine and acts as heterogeneous nidus for crystallization as uric acid binding protein (albumin) binds with calcium oxalate and decreases its solubility (Kalaiselvi *et al.*, 1999). Mp.Eo was found to be effective in lowering uric acid levels. The intoxicated rats were observed for decreased calcium excretion in urine that may be due to the formation of calcium oxalate complex which results in decreased concentration of free calcium (Ca^{++}). Magnesium is a renowned inhibitor of calcium oxalate crystal formation. According to previous findings, Mg-oxalate increases the solubility of calcium-oxalate complex (Tiselius, 2003). Increase in magnesium concentration was observed in Mp.Eo treated rats. Peppermint is rich source of magnesium; therefore, the crystallization inhibitory potential of Mp.Eo, as observed from decreased urinary crystal count and crystal size, may be due to the presence of magnesium. Protein excretion in lithogenic rats was increased that reflects proximal tubular dysfunction. Urinary colloids supersaturation results in precipitation of particles, leading to the subsequent crystallization (Soundararajan *et al.*, 2006). Mp.Eo prevented proteinuria thus possibly could have prevented the nidus formation for crystal nucleation. An increase in urinary phosphate excretion was observed in lithogenic group which along with hyperoxaluria has been reported to provide an environment appropriate for calcium phosphate crystal formation that epitaxially prompts calcium oxalate deposition (Karadi *et al.*, 2006). Mp.Eo lowered the excretion of phosphate and reduced the risk of lithiasis. Impaired renal function in the diseased control group was observed from the markers of tubular and glomerular damage; raised serum creatinine and serum BUN levels, and increase in urinary protein loss, which were significantly reduced in the rats receiving a simultaneous treatment with Mp.Eo. The histological examination revealed that Mp.Eo improved renal epithelial membrane integrity. The improvement in membrane integrity prevented crystal attachment and retention in tubular lumen, thus prevented urolithiasis.

CONCLUSIONS

The results conclude that peppermint essential oil possesses prophylactic and curative antiurolithiatic potential which is possibly due to its antioxidant and anti-inflammatory activities which inhibit organ injury by free radical scavenging effects, thus preventing crystal adhesion and stone formation. The spasmolytic potential of one of its major constituents; menthol, as well as the nephroprotective effects of essential oil, as evident from the normalization of serum creatinine and BUN levels, form a positive correlation regarding its significant potential use in kidney stone disease.

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