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Safety and efficacy of an herbal formulation in patients with renal calculi - A 28 week, randomized, double-blind, placebo-controlled, parallel group study

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ABSTRACT

Background: Urolithiasis is a growing problem worldwide. Many a times, asymptomatic stones are kept under observation. Many herbal preparations are available for the same, but they lack proper scientific documentation.
Objective: To study the anti-urolithiatic effect of an herbal preparation, Subap Plus (IP) capsules in patients with asymptomatic renal calculi of size ranging from 4 to 9 mm.
Material and methods: This was a prospective, randomized, double-blind, placebo-controlled clinical trial conducted in a tertiary care hospital in Pune, India.
Patients with asymptomatic renal calculi of 4–9 mm size were randomized (1:1, block randomization) to one of the group Subap Plus (treatment group) or placebo (placebo group). The study outcome included change in visual analog scale (VAS), change in the surface area and density of calculi and their expulsion. Statistical analysis was performed using student's t-test and Chi-square test.
Results: A total of 120 patients were screened and 84 were enrolled who met the eligibility criteria, of which 65 patients completed the trial (treatment, n = 34; placebo, n = 31). The VAS score significantly decreased in the treatment group (6.9–1.8) than placebo group (7.2–6.8) ($p < 0.001$). The surface area and density were decreased by 47.58% ($p < 0.008$) and 43.01% ($p < 0.001$), respectively, in the treatment group than the placebo group. The expulsion of calculi was significantly higher in the treatment group than placebo group (20.59 vs. 3.23%, $p < 0.03$).
Conclusion: Patients treated with herbal formulation showed better expulsion rate and reduction in surface area and density than the placebo group.

1. Introduction

Urolithiasis is a highly prevalent condition with significant burden on the healthcare system worldwide. The annual incidence of urolithiasis is 0.5% and the lifetime risk of developing urolithiasis is about 10%–15% in the Western world but it can be as high as 20% to 25% in the middle east countries [1]. Collaborative research is essential to address the treatment and prevention of urolithiasis. The majority of kidney stones are asymptomatic at the time of presentation except for acute surgical conditions. Patients with asymptomatic renal calculi often look for alternative medicines. It widely accepted that medicinal plants have played a significant role in various ancient traditional systems of medicine. There is sufficient evidence that herbal extracts have anti-urolithiatic potential. Therapeutic potential of these herbs is also studied by *in vitro* and *in vivo* studies [2].

The increasing use of traditional therapies demands scientifically sound evidence for principles behind these therapies and for the effectiveness of such medicines [3]. Such remedies can be validated at a global level by 'reverse pharmacology' approach. In the concept of reverse pharmacology, safety remains the most important starting point while efficacy becomes a matter of validation [4]. Hence, considering the present scenario, we developed the investigational

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product (IP), an herbal formulation Subap Plus capsules (IP), under our research program. This formulation was prepared from the extracts of *Crateva nurvala* Buch-ham, *Musa x paradisiaca* Linn, *Achyranthes aspera* Linn and *Hordeum vulgare* Linn. All the herbs used in the preparation of Subap Plus capsules are mentioned in Indian traditional system of medicine (Ayurved), [5–8] (https://nccih.nih.gov/research/policies/naturalproduct.htm). In the present study, the safety and efficacy of this formulation were evaluated in patients with asymptomatic renal calculi (4–9 mm). The present study was based on our earlier experience of herbal formulation in the management of urolithiasis [9].

2. Materials and methods

The materials selected from the Ayurvedic literature have the properties like anti-urolithiatic

(*Lekhan* and *Bhedan* of stone material), Diuretic (*Mutral*), Antispasmodic (*Vedanashamak*) and Anti-inflammatory (*Shothagnah*).

2.1. Manufacture of investigational product(IP)

1. Dried stem bark of *C. nurvala* Buch-ham (Three leaved caper/*Varun*), 2. Stem and roots of *Musa x paradisiaca* Linn (Banana/*Kadali*), 3. Whole plant of *A. aspera* Linn (Chaff flower/*Apamarg*) and 4. Seeds of *H. vulgare* Linn (Barley/*Yav*).

The authentication of the raw materials was done at Agharkar research institute, (Department of Science and Technology, GOI) Pune. Voucher specimens of each plant/parts 1). *C. nurvala*- Voucher no.-04/09, 2). *M. x Paradasiaca*- Voucher no. 03/06, 3). *A aspera,* Voucher no.-30/05 and 4). *H. Vulgare,* Voucher no. –16/06/09) has been retained in the research laboratory.

2.2. Method of preparation

C. Nurvala- Preparation of *Varun swaras bhavit Varun churna Varun Bharad* prepared from pulverization of dried stem bark of *Varun*wasmixed with water and boiled (*quath*/ *kadha*/ water decoction). This *quath* was added to *Varun bharad* and kneaded to obtain a mixture of homogenized powder. The product obtained was dried in an oven. This is called *V. swaras bhavit V. churna*. *Kadali swaras bhavit Varun*- Stems of *M. x Paradasiaca* were chopped into pieces and grounded in a mixer to obtain an aqueous extract, which was added to *V. swaras bhavit V. churna*. This was further grounded to make a homogenous composition which was dried in oven to obtain a fine powder. This is called *K. swaras bhavit Varun*. Hence, *K. swaras* was used as *bhavna drvaya* for *Varunchurna* (There are no other terms known to us for *V. swaras bhavit V. churna* and *K. swaras bhavit Varun* that could be cited in traditional literature). While imparting '*bhavana*' the drug is triturated with *swaras* till it gets dried. We have dried it in oven and agree that it should not be done likewise.

M. x Paradasiaca- Banana stems, roots and *kand* were used to prepare *Kadali Kshar* as per herbal guidelines [10].

Acharynthes Aspera- Whole plant of *Apamarg* consisting of flowers,leaves, seeds, roots and fruits (*panchang*) were used to obtain *Apamarg kshar* as per herbal guidelines [10].

Hordeum Vulgare- *Yav* grains are used to prepare *Yavkshar* as per herbal guidelines [10].

Final formulation composition of our formulation was: Each 500 mg capsule contains—*V. swaras bhavit V. churna* (250 mg), *Kadali Kshar* (75 mg), *Yav kshar* (100 mg) and *Apamarg Kshar* (75 mg). (Dosage- One capule- Twice daily, after meals).

Placebo- Identically sized and colored capsules filled with lactose were used in the same dosage so as to match the active ingredient capsule. All this material was manufactured at a GMP (Good manufacturing Practicces) certified facility and was tested in National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory for heavy metal contamination and microbial count.

2.3. Tests involved in standardization process-

The following tests were performed as per herbal pharmacopeia viz. Foreign matter; Organoleptic study – appearance, color, taste and fracture; Macroscopic and microscopic studies; Powder analysis —Ash and extractives and TLC in an accredited laboratory. Laboratory persons were blinded to identify the extract and control capsules. The samples and all the standardization reports of the intermediate and final product have been retained at the manufacturing facility. The staff involved in the study was trained in the evidence based herbal medicine interventions.

The pre-clinical acute and sub-acute safety of this formulation were established in rodents using the Organization of Economic Co-operation and Development (OECD) and U.S. Food and Drug Administration (FDA) guidelines respectively. The acute LD 50 cut off for IP was found to be > 2000 mg/kg in Swiss albino mice and NOAEL was found to be more than 450 mg/kg by oral route for 90 days in Wistar rats [11]. The efficacy of IP was tested and was confirmed in Ethylene Glycol induced rat model (unpublished data).

2.4. Methods

This was a prospective, randomized, double-blind, placebo-controlled clinical trial conducted between September 2010 and December 2013. The trial was registered with Clinical Trial Registry of India with registration number: CTRI/2009/091/000946. The study protocol was approved from Institutional Ethics Committee (IEC), at Ace Hospital and Research Centre, Pune, India. The study was conducted in accordance with the principles that have their origin in the Declaration of Helsinki. Each study participant provided written informed consent before participating in the study. The principal investigator is qualified in both Ayurvedic and allopathic systems of medicine with more than 30 years of experience of and is licensed in India. Both, the principal investigator and enrolled patients were kept blinded about the intervention.

Patients who were asymptomatic at the time of the first visit were screened. Eligible and willing patients were enrolled from the outpatient Department of Urology at Ace Hospital, Pune. Patients visiting the outpatient department with either ultrasound or KUB X-ray (abdomen) reports were also screened for eligibility. In both the cases, those with a renal stone size between 4 mm and 9 mm, underwent non-contrast computerized tomography (CT) scan for confirmation.

Patients with complications and requiring surgical interventions were excluded. Patients with high values of serum. creatinine, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and HbA1c at baseline were also excluded.

Eligible patients were randomized (1:1; block randomization) to one of the treatment group to receive herbal formulation (treatment group) or placebo (placebo group). The randomization, enrollment, assignment and dispensing (medicine/placebo)was done by independent healthcare professionals not involved with the trial. The assessment too was done by them. Enrolled patients received one capsule of 500 mg (either herbal formulation or placebo) twice-a-day after meals for six months. Dosage was

determined from our earlier trial of HERBMED and the dosage mentioned in the traditional Ayurvedic literature as per the nature of the formulation. Heavy metal tests were carried out as per NABL laboratory standards for four metals viz Lead, Cadmium, Mercury and Arsenic. The methods employed for testing these metals were- (AAS) SOP 025 (Pb), SOP 028(Cd), SOP 027(Hg), and SOP 026(As). The concentration of these metals was within acceptable limits.

Qualitative tests were conducted at Indian Drugs Research Association and Laboratory (IDRA and L), Pune. Certificates of analysis have been issued by the laboratory. Samples of *Kadali*, *Yav* and *A. kshar* were analyzed for physical features (color, consistency) loss on drying (in%), acid insoluble ash (in %) and PH(in 10% aqueous solution). The *kshars* were also assayed for-sodium, potassium and iron (in %).

The products obtained from raw herbal materials/extracts, i. e, *V. swaras bhavit Varun churna*and the final formulation (Subap Plus) were analyzed for physical features that included water extractive (in %), alcohol extractive (in %), bulk density (in %) and the particle size too (determined using sieves of varying sizes) apart from the analysis of sodium, potassium and iron (in %).

While TLC (Thin Layer Chromatography) was performed using the following . methodology.

Extraction-Methanol,
Adsorbent -Silica gel G_6OF_{254},
Solvent system-n-Butenol:Acetic acid:water (in 6:1:2),

Detection—UV 254 nm, UV 365 nm, and Anisaldehyde Sulfuric acid Reagent.

The samples and all the standardization reports of the intermediate and final product have been retained at the manufacturing facility.

Blood sample for biochemical evaluation of complete blood count, blood sugar levels, serum creatinine, blood urea nitrogen, serum electrolytes, serum bilirubin (total), SGPT, SGOT, ALP, total proteins, HbA1c and urine (routine and microscopic) was performed at baseline and at the end of the study. Follow-up was done every month for pain and expulsion of calculi if any. No rescue medicine was provided during follow-up. Patient was asked to take tablet diclofenac sodium 50 mg (as necessary or twice-daily) in case of severe pain. The CT scan was done at the start of the study and also when the patient provided the history of expulsion or at the end of the study.

The clinical assessment for pain was analyzed by visual analog scale (VAS), on a scale of 0—10. Expulsion and change in the size and density of calculi were assessed by CT scan. Size of calculi was calculated by the surface area of the stone on the basis of length and width (Guidelines on Urolithiasis: European Association of Urology, 2001). In case of multiple calculi, the cumulative diameter (mean value of multiple stones) was considered for calculation of surface area. Density was calculated by considering the largest length and width intersection point to avoid error. Density was measured in Hounsfield unit threshold by CT scan.

2.5. Statistical analysis

Sample size and dropout rate were determined from our earlier study done on the same condition and other trials completed on medical management of urolithiasis. In our opinion this being a non-critical minor medical condition there is a tendency for such a large drop out. Generally, there has been a 30% drop out in our other trials. The results were analyzed by student t-test and Chi-square test using Graph Pad Software Inc, 2003. The confidence interval was considered at 95% ($p < 0.05$).

3. Results

A total of 120 patients were screened, of which 84 patients were randomized (treatment group, n = 42; placebo group, n = 42). Of those enrolled, 65 completed the study (Fig. 1); 19 (22.62%) patients dropped out (treatment group; n = 8 and placebo group; n = 11). In the treatment group, three patients required surgical intervention, while five patients were not considered for analysis due to irregular follow-up. In the placebo group, eight patients withdrew their consent and three patients required intervention due to severe abdominal pain and obstructive pelvicalyceal system (Fig. 1).

The baseline characteristics were comparable for both the groups. The mean (SD) age was 39.24 (13.3) years at the time of study inclusion and the male to female ratio was 2:1. Of the 65 randomized patients, 37 (56.92%) patients had a single stone, while 28 (43.08%) patients had multiple or bilateral calculi. Hemogram and other biochemical parameters did not change significantly in either of the group after the study period.

3.1. Parameter-wise comparisons

The change in mean (SD) surface area from baseline was significantly reduced in the treatment group from 27.6 (15) sq. mm to 21.7 (13.2) sq. mm ($p < 0.0243$); however, the change was not significant in placebo group (31.5 [16.5] sq. mm to 32 [16.1] sq. mm; $p = 0.28$). The difference between the two groups was statistically significant ($p < 0.005$) (Table 1).

The mean (SD) stone density reduced from 834.3 (357.3) HU to 740.9 (338) HU ($p = 0.062$) in the treatment group; however, it was increased in the placebo (901.9 [314.7 HU to 1059.6 [405.9] HU; $p = 0.008$). The difference between the two groups was statistically significant ($p < 0.001$) (Table 1).

In placebo group, only one of 31 (3.2%) patients had stone expulsion, while in treatment group, seven (20.6%) patients had stone expulsion ($p = 0.03$). In treatment group, the mean (SD) VAS score reduced from 6.9 (1.6) to 1.8 (0.9) ($p < 0.0001$) and in placebo group, the VAS score reduced from 7.2 (1.5) to 6.8 (1.5) ($p < 0.161$). The difference between the two groups was statistically significant ($p < 0.0001$).

No major adverse events were noted in both the groups. In the treatment group, three patients had an episode of belching after consuming medication on empty stomach, which settled down when the medication was taken after meals. In the placebo group, two patients had nausea.

4. Discussion

The increase in the number of patients of urolithiasis worldwide is truly a cause of concern [12]. A very common and uncomplicated disease at an early phase, may at times lead to kidney failure. One of the reasons could be the change in the biological clock as a result of modernization. Currently, several minimally invasive options are available for the management of symptomatic renal stones and for surgical indications. However, residual stones/fragments remain a concern. The recurrence rate of kidney stones without preventive treatment is approximately 10% at 1 year, 33% at 5 years and 50% at 10 years [13]. Similarly, the term ‘clinically insignificant’ is probably a misnomer, about half of the patients with a stone of <4 mm will have symptoms and require intervention or both, within 5 years [14]. In another study by Burgher et al., which involved patients with asymptomatic stones, around 77% of patients showed disease progression, with 26% requiring intervention with a mean follow-up period of 3.26 years [15]. In the same study, Kaplan—Meier

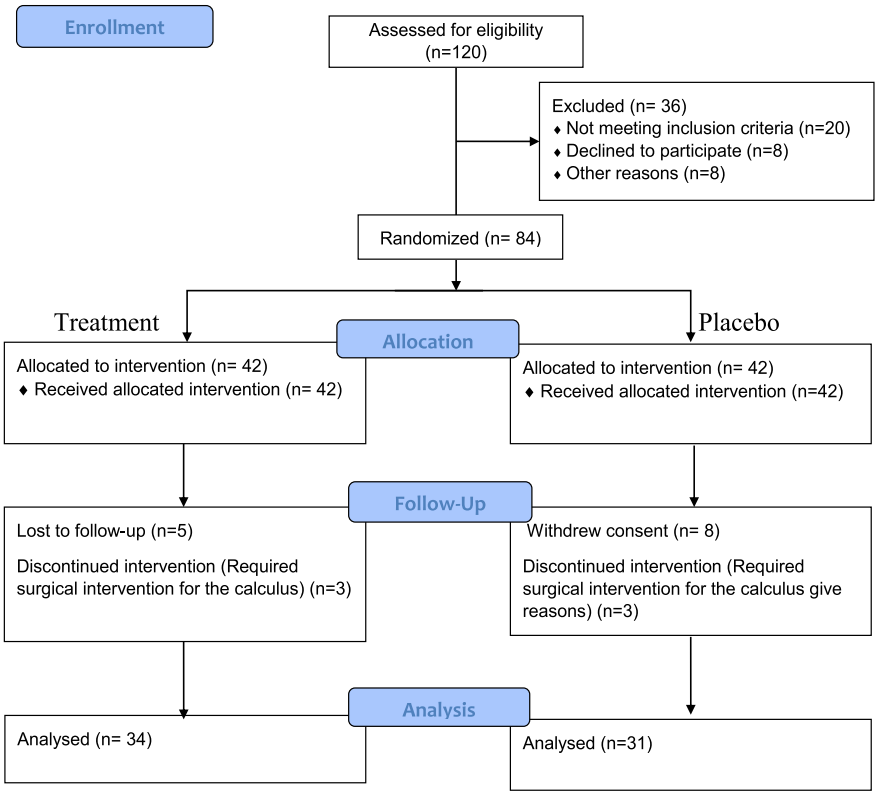

Fig. 1. CONSORT flow diagram.

analysis suggested 50% of patients required intervention at 7 years [15].

Hubner and Porpaczy analyzed the course of 80 'stone periods' in 62 patients. Patients were followed-up for an average period of 7.4 years and divided into 2, 5 and 10 years groups according to the length of follow-up. During these intervals, 55%, 79%, and 38% of patients, respectively, reported infection or flank pain [16]. Hence, asymptomatic patients undergo intervention to prevent future stone associated events [15]. Collins and Keely concluded that the use of shock wave lithotripsy for asymptomatic calyceal stones did not improve the clinical outcome [17]. Hence, the decisions to proceed with shockwave lithotripsy should be considered carefully with sound clinical evidence and based on clear intent to avoid likely complications [15].

The non minimally invasive treatments do not avoid the possibility of new stone formation [18]. Various therapies including thiazide diuretics and alkali–citrate is being used to prevent recurrence of hypercalciuria and hyperoxaluria induced calculi, but scientific evidence for their efficacy is less convincing [19]. Targeted medical therapies were used to treat asymptomatic renal calculi by Burgher et al. [15]. While the incidence of stone growth was lower in the population receiving medical therapy, the difference was not statistically significant [15]. The incidence of adverse events is quite significant in the currently available medical therapies. Compliance for the treatment is also poor in long-term duration. In a study conducted for medical prophylaxis for stone disease, Pak and others found that nearly half the patients who were prescribed potassium or magnesium for effective prophylaxis did not take the drug continuously for 3 years [20]. Adverse reactions attributed to the study medication accounted for 10.1 % patients who stopped taking potassium or magnesium citrate. In another study, where potassium citrate was used for long-term prophylaxis in calcium nephrolithiasis, the incidence of gastrointestinal adverse events ranged from 9% to 17% [21]. In a recent meta-analysis, alpha blockers have shown a significant role in medical expulsive therapy [22].

In our earlier study, an herbal composition 'Herbmed' (H), with two ingredients - showed significant 33% reduction in the calculus sized between 5 and 10 mm, and 11.25% reduction in calculus of size >10 mm [9]. Based on the above study, the present trial was

Table 1
Summary of stone morphology.

Parameters	Placebo group N = 31			Herbal treatment group N = 34		
	Pre-treatment	Post-treatment	P value	Pre-treatment	Post-treatment	P value
Length (mm)	6.80 (1.99)/7.5	7.02 (2.11)/7.5	–	6.12 (1.95)/6.45	5.39 (2.14)/5.75	–
Width (mm)	5.43 (1.83)/5.6	5.44 (1.74)/5.2	–	5.38 (2.02)/5.4	4.64 (1.76)/4.75	–
Surface Area (mm^2)[a]	31.47 (16.46)/33.17	32.04 (16.05)/30.37	0.2870	27.58 (14.95)/27.59	21.70 (13.19)/21.38	0.0243
Density (HU)[b]	901.94 (314.68)/921	1059.55 (405.93)/1108	0.0008	834.32 (357.31)/830.5	740.88 (338.04)/722.5	0.0624

Data presented as mean (standard deviation)/median. HU, Hounsfield Units.
[a] Post-treatment $p < 0.005$ between placebo versus herbal treatment group.
[b] Post-treatment $p < 0.001$ between placebo versus herbal treatment group.

designed with calculus size ranging from 4 to 9 mm. Also, to increase the potency of H, as mentioned earlier, two more ingredients were added to formulate IP. In our earlier study, treatment period was only 3 months and was stated that the medicine should be given for a longer duration to get promising results. Hence, to overcome the limitations of our previous study, in the present study a treatment period of 6 months was considered.

The IP contains four active ingredients: *C. nurvala, M. x paradisiaca, A. aspera and H. vulgare.C. Nurvala* contains active constituent Lupeol, which is very well known for its anti urolithiatic activity through anti-oxaluric and anti calciuric effect [23]. The decoction of *C. Nurvala* in experimental urolithiasis showed regulatory action on endogenous oxalate synthesis [24]. The reversal of increased urinary excretion of the crystalline constituents is done along with lowered magnesium excretion by *C. Nurvala*. This action might be mediated through (Na+, K+) ATPase affecting the transport mechanism [25].

In urolithiasis induced by Ethylene glycol in rats, use of Musa tablet showed a significant decrease of the elevated level of oxalate, calcium, and phosphate in urine; increase in urine volume and reduction in deposition of oxalate crystals in renal tubules [26]. The extract of Musa reduced urinary oxalate, glycolic and glyoxylic acid and phosphorus excretion in hyper oxaluric rats [27]. Another action of Musa showed that the Ethylene Glycol induced rise of calcium and oxalate crystals in urine was reduced [28], possibly due to binding of oxalate to form soluble complexes. This mechanism of banana extract has shown a significant lowering of GAO (Glycolic Acid Oxidase) in liver tissue in hyperoxaluric rats [29].

Extract of *A. aspera* has effective inhibitory action on CaOx nucleation and growth *in vitro* and exhibited the reduction in oxalate induced injury on epithelial cells, NRK52E [30]. It also prevents super saturation, decreases crystal size and thus facilitating early expulsion [31]. Reduction in nucleation increases the meta stable limit of oxalate in urine [32]. Chemical constituents like higher carboxylic acids such as citrate, chelates the calcium and form a soluble salt which is excreted in urine [32]. The macromolecule of a higher molecular weight of plant extract exerts their action similar to natural urinary inhibitors and inhibits crystal aggregation and growth [32,34].

Another constituent *H. Vulgare* has anti-inflammatory and similar actions like other constituents as above in lowering the rate of stone forming constituents and increase in urinary citrate excretion [33]. The pathogenesis of calcium oxalate stone is a multistage procedure and in essence includes Nucleation>>>>-Crystal growth>>>>Crystal aggregation >>>>Crystal retention [33]. The plant extracts used in our formulation act at various levels of stone formation as mentioned above [34]. The results obtained in our study, reduction in pain score, higher expulsion rate, reduction in surface area and density can be explained on the basis of the above mechanism of action.

In our animal studies, we have seen significant restorative activity on injury caused to renal cells due to crystal deposition, which we call as 'renoprotective function'. It is an important observation, because cellular injury may be even more important determinant in the promotion and progression of kidney stones [34]. The author D. K. Basavraj and others state that regulation of inflammation may lead to new attractive therapeutic strategies for the management of stone disease.

To the best of our knowledge, this is the first study on herbal extract in the management of urolithiasis, where preclinical safety, efficacy, standardization of the product including heavy metal, microbial testing and randomized, double-blind trial were conducted. The mechanism of action is also proposed based on modern references and references in the treatises.

However, authors acknowledge a few limitations of this study. The sample size was small and the duration of the study was short. We did not analyze expelled stones. Also, detailed metabolic workup before and after the medication could have helped in deducing the role of action of the plant extracts. In fact, in our other study (unpublished data) the formulation has shown favorable effects on hypercalciuria, hyperoxaluria, correction of hypocitratiuria, hypomagnesiuria and supersaturation index.

5. Conclusion

In this exploration, the IP is exhibiting promising anti-urolithiatic and analgesic activity due to the synergistic effect of its ingredients without any adverse events. The IP has reduced the size and density of renal calculi, thereby helping in their expulsion. It has also helped to reduce the pain caused by renal stones as compared to placebo. Possibly, this could be a drug of choice in future for small, non-obstructive renal calculi and in the prevention of recurrence of renal stones as well.

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Conflicts of interest

First author holds the patent for the investigational product. He is working as the chairperson of the Department of Urology at Ace Hospital and Research Centre, Pune, India. Among other authors, Dr. A. M. Mujumdar was director of the Department of Research in Ace Hospital, Dr. Bernard Fanthome is presently the medical director at Ace Hospital while Dr. Supriya Phadke is an ex-employee of Department of Research, Ace Hospital.

We wish to confirm that the first author/corresponding author is the patent holder for this formulation and there has been no significant financial support for this work that could have influenced its outcome.

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