ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 8, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ANTIUROLITHIATIC PROPERTY OF ETHANOLIC EXTRACT OF FENNEL SEEDS IN MALE WISTAR ALBINO RATS

BASVARAJ POOJAR[1], BALAJI OMMURUGAN[1], SHALINI ADIGA[1]*, HUBAN THOMAS[2]

[1]Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India. [2]Department of Anatomy, Kasturba Medical College, Manipal University, Manipal, Karnataka, India. Email: Shalini.adiga@manipal.edu

Received: 04 April 2017, Revised and Accepted: 15 May 2017

ABSTRACT

Objective: Few studies have explored the diuretic property of fennel (*Foeniculum vulgare*). Hence, the aim of this study was to evaluate the antiurolithiatic property of ethanolic extract of fennel seeds in male Wistar albino rats.

Methods: Prophylactic and curative urolithiasis models were used with 5 groups of 6 rats in each model. Ethanolic extract of fennel seeds in three doses 100, 200, and 300 mg/kg was used. Cystone 750 mg/kg was used as a standard drug. All drugs were administered orally. Zinc discs were surgically implanted in the bladder in all rats. After recovery, rats in the prophylactic model received three different doses of ethanolic extract of fennel seeds along with 1% ethylene glycol for 2 weeks whereas the rats in the other model received 1% ethylene glycol for 2 weeks followed by an ethanolic extract of fennel seeds in three doses for the next 2 weeks. Both models had a control group receiving 1% ethylene glycol. At the end of study period, rats were sacrificed and vesical calculi collected, weighed, and statistically evaluated using one-way ANOVA.

Results: In both the models, all three doses of an extract of fennel seeds were effective in reducing stone formation as compared to control group with $p<0.05$. In both the models, all three test doses were comparable with cystone, but 300 mg/kg extract in prophylactic showed significance ($p <0.05$) when compared to standard.

Conclusion: Fennel seeds can be used prophylactically as well as curatively in the treatment of urolithiasis. However, further studies and clinical trials are warranted to explore this property.

Keywords: Zinc discs, Ethylene glycol, Urolithiasis, Fennel seeds.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18923

INTRODUCTION

Uroliths or calculi, usually known as urolithiasis is a multifactorial disease having a myriad of etiologies such as biochemical, metabolic, epidemiological, socioeconomic, drug–induced, and genetic factors affecting any part of urinary system. It is the third most common urological disease condition affecting people globally since antiquity [1-4]. Urolithiasis is said to be a recurrent disease state with a recurrence rate of about 98% in 25 years from the time it is initially reported [5]. It has a global incidence of 1%, prevalence of 3-5% with 15-25% lifetime risk [6]. It has an incidence of 10-13% in industrialized countries with 1-5% incidence in Asia [7]. It affects men more than women with the highest prevalence in age groups around 20-40 years [8]. Uroliths can lead to life-threatening complications such as acute renal failure, chronic renal failure, pyelonephritis, and perinephric abscess, and add to morbidity and mortality [9].

Urolithiasis is a consequence of complex physiochemical processes, and the major contributory factors are urinary supersaturation, crystallization, calculogenesis, and matrix formation. Various types of stones include calcium oxalate stones, struvite stones, uric acid stones, and cysteine stones with CaOx stones having the highest incidence of 75% in contrast to cysteine stones having incidence of 1-2% [10]. Various risk factors include hypercalciuria, hypercalcemia, hyperuricosuria, dehydration, anatomical abnormalities, increased urinary pH, and various drugs such as sulfonamides, corticosteroids, fluoroquinolones, tetracycline, and indinavir [11]. The imbalance between promoter and inhibitors of crystallization compounds, formation of Randall Plaques, conversion of urea into carbon dioxide and ammonia by microorganisms leading to increase in pH, the extra tubular theory/intratubular theory of stone formation, various proteins like nephrocalcin, Tamm–Horsfall protein are implicated in the pathogenesis of uroliths [11].

Medical treatment includes acute pain management with nonsteroidal anti-inflammatory agents, chronic stone management using thiazide diuretics, potassium sparing diuretics, calcium channel blockers, allopurinol along with alkalization of urine, and increase in the fluid intake. When medical management fails to expel the stones usually of size >5 mm, surgical interventions such as extracorporeal shock wave lithotripsy, laser beam ureteroscopy, percutaneous nephrolithotomy, and open surgeries are indicated [11].

Phytotherapeutic agents are claimed to be an alternative in treating urolithiasis by various mechanisms which include antimicrobial, antioxidant, diuresis, and increased urinary citrate excretion. Many herbal plants have been tested *in vitro*, *in vivo* and in clinical experiments for their urolithiatic property [11].

Foeniculum vulgare (Fennel) is a widely acclaimed medicinal plant used to treat more than 40 medical conditions. *F. vulgare* contains various substances, namely, phenolic compounds, fatty acids, amino acids, flavonoids, and volatile compounds which are responsible for various medicinal properties. Literature evidence with compilation of various data shows the antioxidant, antimicrobial, antiviral, antiplatelet, antithrombotic, and anticancer properties of fennel [12]. Fennel seeds were proven to be an effective renoprotective agent in an animal study conducted on polycystic ovary syndrome rats [13]. The database search shows there is no report on *in vivo* study using *F. vulgare* for urolithiasis. Hence, our study was aimed to evaluate the antiurolithiatic property of *F. vulgare* using ethylene glycol as a urolithiatic agent in male Wistar rats.

METHODS

The study commenced after approval by Institutional Animal Ethics Committee, (IAEC/KMC/14/2016).

Chemicals, drugs, and instruments

Lithogenic agent ethylene glycol (Merck, India) and standard drug cystone (Himalaya Herbal Healthcare, Bengaluru, India) were used in this study.

Plant preparation and extraction

The seeds were purchased from the local market, and its authenticity confirmed from the botanist Mahatma Gandhi Memorial College, Udupi. The seeds were made into fine particles using an electrical grinder to increase the surface contact with a solvent in the extraction process. The powder obtained was bagged into thimble by cellulose, which was then placed in the Soxhlet instrument for extraction. Extraction solvent (ethanol) 90%, was boiled in the round bottom flask at 40°C for 24 hrs, then essence was subjected to evaporation in a water bath to take out ethanol. The obtained yield was measured (10%) and preserved in desiccator till further use at room temperature [14].

Animals

Male albino rats of Wistar strain weighing 200-250 g were used for the study. The animals were caged under standard condition, 12:12 light-dark cycle, 50% humidity and 28°C and provided with standard food granules and water *ad libitum*.

Urolithiasis model

Preclinical zinc pellet implantation model was used for evaluation of the antiurolithiatic activity [15]. Pre-weighed zinc pellets were placed in the urinary bladder of rats. Preoperatively the rats were fed with 5 ml of water orally to dilate the urinary bladder for apparent identification. Animals were anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg) combination through intraperitoneal route. The surgical site was shaved and disinfected with alcohol. After making a suprapubic incision and opening the abdominal cavity, the urinary bladder was exposed. A small horizontal nick was made at the apex of the bladder. Zinc pellets weighing 22 mg were placed inside the bladder. After placing zinc pellets bladder was closed with 1-2 dissolvable chromic catgut (4-0), followed by closure of abdominal cavity. The skin was sutured with, braided sterile silk thread. All rats were subjected to a post-operative recovery period of 1 week with standard care, food, and water given *ad libitum*.

Three doses of *F. vulgare* seed extract, 100 mg/kg, 200 mg/kg, and 300 mg/kg were selected based on literature reports on its toxicity studies in rats [12].

Procedure of study

Ethanolic extract of fennel seeds as test drug was evaluated for antiurolithiatic activity *in vivo*, with three different doses 100 mg/kg, 200 mg/kg, and 300 mg/kg and these extracts were compared with normal control group and rats dosed with standard herbal formulation cystone.

The prophylactic effect of fennel seeds extract against surgically induced urolithiasis was tested in a total of 30 rats divided into 5 groups of 6 animals per group. After recovery from zinc implantation surgery, rats were dosed as follows:

Group 1: Control group was administered 1% ethylene glycol solution (lithogenic agent) in drinking water *ad libitum* for 2 weeks.

Group 2: Standard treatment group was treated with cystone 750 mg/kg by oral gavage along with 1% ethylene glycol solution *ad libitum* for 2 weeks.

Group 3: Fennel seed extract was administered at a dose of 100 mg/kg by oral gavage daily for 2 weeks along with 1% ethylene glycol in water *ad libitum*.

Group 4: Fennel seed extract was administered at a dose of 200 mg/kg by oral gavage daily for 2 weeks along with 1% ethylene glycol in water *ad libitum*.

Group 5: Fennel seed extract was administered at a dose of 300 mg/kg by oral gavage daily for 2 weeks along with 1% ethylene glycol in water *ad libitum*.

The curative effect of fennel seeds extract against surgically induced urolithiasis in Swiss albino Wistar rats was tested in a total of 30 rats divided into 5 groups of 6 animals per group. After recovery from zinc implantation surgery rats was dosed as follows:

Group 1: Control group was administered 1% ethylene glycol in water *ad libitum* for 2 weeks followed by 2 weeks' water.

Group 2: Standard group animals received lithogenic agent 1% ethylene glycol in drinking water for 2 weeks followed by herbal formulation cystone 750 mg/kg period of 2 weeks orally.

Group 3: Test group treated with 1% ethylene glycol in drinking water for 2 weeks followed by 2 weeks of 100 mg/kg test drug given orally.

Group 4: Test group treated with 1% ethylene glycol in drinking water for 2 weeks followed by 2 weeks of 200 mg/kg test drug given orally.

Group 5: Test group treated with 1% ethylene glycol in drinking water for 2 weeks followed by 2 weeks of 300 mg/kg test drug given orally.

At the end of the treatment period of 2 weeks for prophylactic and 4 weeks for the curative model, animals were sacrificed and vesicle calculi collected, weighed, and statistically evaluated.

Weight of vesicle calculus

The weight of vesicle calculi was determined by removing the zinc disk and adhered crystals. Removed discs were air dried and separately stored in polythene bags. The difference in weight between initial weight of zinc pellet and final weight of zinc pellet with stone embedded indicate the amount of stone formed.

Histopathology of kidney

The animals were sacrificed after treatment period by an excess dose of urethane. Bilateral kidney tissues were collected for histological examination. Collected tissues were preserved in 10% v/v formalin till histological section was made. Kidneys were sectioned into 5 μm thin and were enclosed in paraffin, stained using Hematoxylin and Eosin, and examined under optical light binocular microscope in ×10 microscopic field and pictures were captured using Olympus microscope camera.

Statistical analysis

All data were statistically analyzed using one-way ANOVA followed by *post hoc* analysis. $p<0.05$ was taken as statistical significance.

RESULTS

A. In the prophylactic urolithiatic model as in Table 1, the cystone group, 100 mg/kg fennel, 200 mg/kg fennel, and 300 mg/kg fennel showed a significant reduction in crystal deposition around the implanted zinc discs when compared to control group. There was no intragroup significance between the three test groups. When compared to standard group cystone, 100 mg/kg fennel (p=0.402) and 200 mg/kg fennel (p=0.147) did not show any difference but 300 mg/kg (p=0.041) showed significant difference. Stones formed in all the rats are shown in Fig. 1. In this model, one animal died during the study period following surgery in each group, and a total number of animal in each group was five.

Table 1: Prophylactic activity of extarct *Foeniculum vulgare* seeds on crystal deposition around zinc disc

Groups (n=5)	Mean stone weight (mg)±SEM	p value
Control	0.344±0.023	-
Standard	0.261±0.036	<0.001*
T100	0.237±0.009	<0.001*
T200	0.229±0.002	<0.001*
T300	0.220±0.015	<0.001*, <0.05**

Number of rats (n)=5. *p value significant when compared to control group. **p value significant when compared to standard cystone group. SEM: Standard error of the mean

B. In the curative model, as in Table 2, standard drug cystone, 100 mg/kg fennel, 200 mg/kg, and 300 mg/kg showed a significant reduction in crystal deposition when compared to control group. All three test groups showed no statistical difference when compared to standard drug cystone. Intragroup analysis showed 200 mg/kg dose (p=0.009) and 300 mg/kg dose caused a significant reduction in crystal deposition when compared to 100 mg/kg group. There was no significant difference between 200 mg/kg and 300 mg/kg dose. All stones formed are shown in Fig. 2.

Histopathological assessment

In both prophylactic model and curative model, the control groups showed calcium oxalate crystal deposition along with necrotic changes. It also showed leukocyte infiltration along with tubular dilation, swelling and damage. In standard and all the three test groups of both the prophylactic and curative model, histopathology of kidney was normal without any changes noted as depicted in Figs. 3 and 4.

DISCUSSION

This study evaluated the antiurolithiatic activity of ethanolic extract of seeds of *F. vulgare* in normal albino Wistar rats. The most commonly used model for inducing urolithiasis in rats is zinc disc implantation model along with ethylene glycol because in this model there is no severe renal damage and it also mimics the human etiology of stone formation [16]. Literature evidence says the administration of ethylene glycol following zinc disc implantation in bladder leads to deposition of calcium oxalate crystals [17].

In our study in the prophylactic model, 100 mg/kg, 200 mg/kg, and 300 mg/kg *Foeniculum* extract showed significant reduction in stone formation when compared to untreated control group and the antiurolithiatic effect of *F. vulgare* was also comparable with standard 750 mg/kg cystone when used at dose of 100 mg/kg and 200 mg/kg but 300 mg/kg dose showed significance when compared to cystone. Hence, this proves the antiurolithiatic property of *F. vulgare* when used prophylactically in the treatment of urolithiasis. The mechanism of inhibition of stone formation may be attributed to its diuretic property when used prophylactically [18].

In our study in the curative model, all three doses of *F. vulgare* were helpful in reducing the stone size when compared to the control group, and the antiurolithiatic activity of all the test dose extracts was comparable to standard drug cystone. Among the three doses, 300 mg/kg and 200 mg/kg showed a better reduction in stone size when compared

Table 2: Curative activity of extract *Foeniculum vulgare* seeds on crystal deposition around zinc disc

Groups	Mean stone weight (mg)±SEM	p value
Control	0.427±0.029	-
Standard	0.300±0.045	<0.001*
T100	0.348±0.016	<0.01*
T200	0.280±0.042	<0.001*, <0.05**
T300	0.254±0.010	<0.001*, <0.001**

Number of rats in each group (n) is 6. *p value significant when compared to control group. **p value significant when compared to 100 mg/kg fennel. SEM: Standard error of the mean

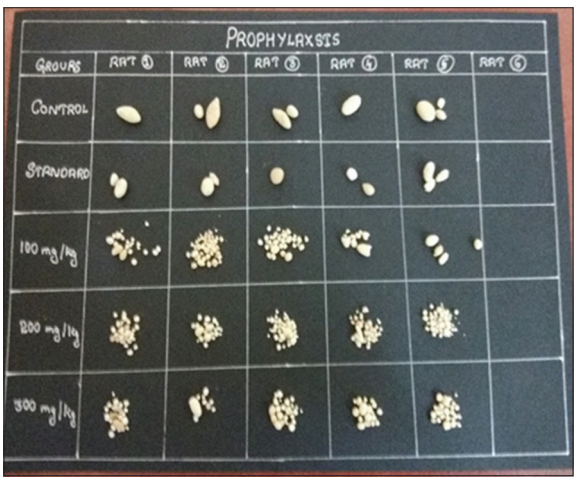

Fig. 1: Images of stones in prophylactic model

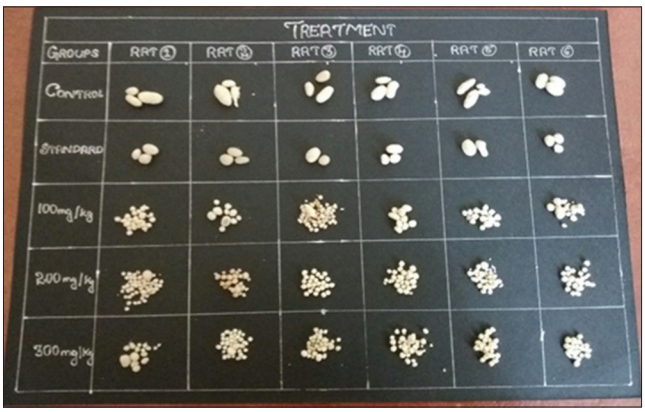

Fig. 2: Images of stones in curative model

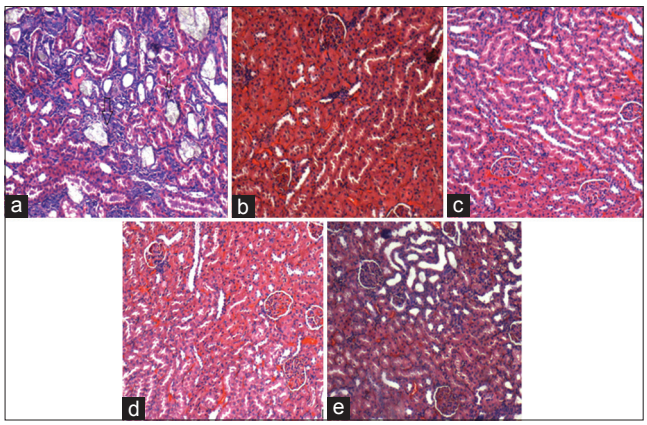

Fig. 3: Histopathology of kidney tissue in prophylactic model. (a) Control group, (b) standard group, (c) test dose 100 mg/kg extract, (d) 200 mg/kg extract, and (e) 300 mg/kg extract

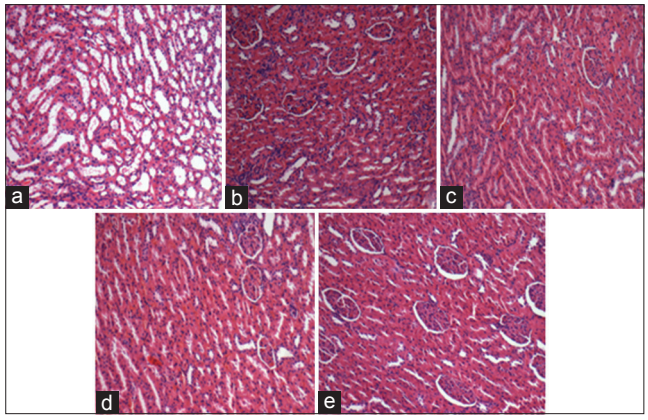

Fig. 4: Histopathology of kidney tissue in curative model. (a) Control group, (b) standard group, (c) test dose 100 mg/kg extract, (d) 200 mg/kg extract, and (e) 300 mg/kg extract

to 100 mg/kg. There was no significant difference between 200 and 300 mg/kg dosage. Mechanisms that can be attributed to the curative urolithiatic effect of *F. vulgare* is the diuretic [18] as well antioxidant property of the plant [12]. Oxalate crystals are mainly deposited and leads to crystallization due to oxidative stress [19,20]. Phytochemical analysis has shown that *F. vulgare* contains certain volatile compounds, flavonoids, phenols, and various fatty acids [12]. Some studies have shown that these compounds possess antiurolithiatic property due to its antioxidative nature [21,22].

CONCLUSION

Our study was the first study to explore the antiurolithiatic property of *F. vulgare* seed extract. A dose-dependent antiurolithiatic effect was seen. All three doses of fennel seeds were equally efficacious as standard drug cystone in curative model, whereas 300 mg/kg dose showed better effect than standard cystone treatment when used prophylactically. Hence, further clinical trials are needed to evaluate the antiurolithiatic property of *F. vulgare* in human patients, and appropriate antiurolithiatic dosages can be ascertained. Urolithiasis, being a recurrent problem globally and with medical expulsion therapy having its own drawbacks with respect to stone size in consideration, alternative medicine in the form of herbal products are the current topic of research and the need of the hour. More focus should be thrown on preclinical as well as clinical research using herbal plants in the treatment of urolithiasis.

REFERENCES

1. Divakar K, Pawar AT, Chandrasekhar SB, Dighe SB, Divakar G. Protective effect of the hydro-alcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats. Food Chem Toxicol 2010;48(4):1013-8.
2. Bouanani S, Henchiri C, Migianu-Griffoni E, Aouf N, Lecouvey M. Pharmacological and toxicological effects of *Paronychia argentea* in experimental calcium oxalate nephrolithiasis in rats. J Ethnopharmacol 2010;129(1):38-45.
3. Alessandra CP, Elvino JG. Dietary calcium intake among patients with urinary calculi. Nutr Res 2003;23:1651-60.
4. Osborne CA, Lulich JP, Swanson LL, Albasan H. Drug-induced urolithiasis. Vet Clin North Am Small Anim Pract 2009;39(1):55-63.
5. Lewandowski S, Rodgers AL. Idiopathic calcium oxalate urolithiasis: Risk factors and conservative treatment. Clin Chim Acta 2004;345(1-2):17-34.
6. Moe OW, Pearle MS, Sakhaee K. Pharmacotherapy of urolithiasis: Evidence from clinical trials. Kidney Int 2011;79(4):385-92.
7. Saha S, Verma RJ. Inhibition of calcium oxalate crystallisation *in vitro* by an extract of *Bergenia ciliata*. Arab J Urol 2013;11(2):187-92.
8. Senthy S, Christoper F. The metabolic basis of urolithiasis. Surgery 2008;26(4):136-40.
9. Hussain M, Rizvi SA, Askari H, Sultan G, Lal M, Ali B, *et al.* Management of stone disease: 17 years experience of a stone clinic in a developing country. J Pak Med Assoc 2009;59(12):843-6.
10. Das I, Gupta SK, Ansari SA, Pandey VN, Rastogi RP. *In vitro* inhibition and dissolution of calcium oxalate by edible plant *Trianthema monogyna* and pulse *Macrotyloma uniflorum* extracts. J Cryst Growth 2005;273:546-54.
11. Sodimbaku V, Pujari L. Urolithiasis-an updated review over genetics, pathophysiology and its clinical management. Int J Pharm Pharm Sci 2014;6(11):23-31.
12. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int 2014;2014:842674.
13. Mazaheri S, Nematbakhsh M, Bahadorani M, Pezeshki Z, Talebi A, Ghannadi AR, *et al.* Effects of fennel essential oil on cisplatin-induced nephrotoxicity in ovariectomized Rats. Toxicol Int 2013;20(2):138-45.
14. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants 2015;4(3):3-8.
15. George MM, Adiga S, Avin S, Tripathy A. Evaluation of diuretic and antiurolithiatic properties of ethanolic extract of *Sida acuta* Burm F. In wistar albino rats. Int J Pharm Pharm Sci 2016;8(5):122-6.
16. Vyas N, Argal A. Antiurolithiatic activity of extract and oleanolic acid isolated from the roots of *Lantana camara* on zinc disc implantation induced urolithiasis. ISRN Pharmacol 2013;2013:951795.
17. Khan SR. Animal models of kidney stone formation: An analysis. World J Urol 1997;15(4):236-43.
18. Bekhradi R. New Treatment Plant. 1st ed. Iran: Bekhradi Publication; 2004. p. 61-73.
19. Pearle MS, Lotan Y. Urinary lithiasis: Etiology, epidemiology, and pathogenesis. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. Campbell-Walsh Urology. 10th ed. Philadelphia, PA: Saunders-Elsevier; 2012. p. 1257-86.
20. Miller C, Kennington L, Cooney R, Kohjimoto Y, Cao LC, Honeyman T, *et al.* Oxalate toxicity in renal epithelial cells: Characteristics of apoptosis and necrosis. Toxicol Appl Pharmacol 2000;162(2):132-41.
21. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63(7):1035-42.
22. Kähkönen MP, Heinonen M. Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 2003;51(3):628-33.