ORIGINAL ARTICLE

Antilithiatic effect of flowers of *Jasminum Auriculatum* Vahl

Yogendr Bahuguna, Mohan Singh Maniyari Rawat[1], Vijay Juyal[2], Vikas Gupta
Division of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun – 248 001, Uttarakhand, [1]Department of Chemistry, H.N.B. Garhwal University, Srinagar, Pauri Garhwal, Uttarakhand, [2]Department of Pharmacy, Kumaun University, Bhimtal Campus, Bhimtal, Nainital, Uttarakhand, India

The effect of oral administration of aqueous and alcohol extracts of *Jasminum auriculatum* Vahl (Oleaceae) flowers on calcium oxalate nephrolithiasis has been studied in male albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Supplementation with aqueous and alcohol extract of *J. auriculatum* flowers significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by curative and preventive treatment using aqueous and alcohol extracts. The results indicate that the flowers of *J. auriculatum* are endowed with antiurolithiatic activity.

Key words: Ethylene glycol, flowers, hyperoxaluria, *Jasminum auriculatum,* nephrolithiasis

INTRODUCTION

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones.[1]

A number of plant drugs have been used in India and elsewhere which claim efficient cure of urinary stones.[2] *Jasminum auriculatum* Vahl (Oleaceae) commonly known as Juhi, Needle flower jasmine, Yutika, grows almost throughout South India, on the dry slopes of the Western Ghats. *J. auriculatum* has been claimed in traditional literature to be valuable against a wide variety of diseases. Indian Materia Medica describes the use of flowers of *J. auriculatum* in the treatment of a number of ailments, including burning sensation, diuretic, hyperdesia, ulcers, odontalgia, stomatopathy, ophthalmopathy, cardiopathy, urolithiasis, nephrolithiasis, strangury and dermatopathy.[3]

In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of *J. auriculatum* flowers extracts using ethylene glycol induced hyperoxaluria model in rats.

MATERIALS AND METHODS

Preparation of Extract

The fresh flowers of *Jasminum auriculatum* Vahl were collected from local areas of Belgaum, Karnataka, India during May-2008 and authenticated at Botanical Survey of India (BSI), Dehradun, India. A voucher specimen of the plant was deposited in the Botanical Survey of India herbarium under the number BSI/DD/Tech/572.

The flowers were dried in shade and were ground to get a coarse powder (40 mesh size). The aqueous extract (AqE, 10%, w/v) of dried flowers was prepared using chloroform water, i.p., by maceration method for 7 days at room temperature (yield 8.6%, w/w) and alcohol extract (AlcE, 10%, w/v) of dried flowers was prepared using 70% (v/v) alcohol by soxhlet method at a temperature of 60-70°C (yield 5.4%, w/w). The extracts were then filtered, concentrated under vacuum and freeze-dried.

Pharmacological Screening for Antiurolithiatic Activity

Animal Selection

Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between 150 and 200 g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with

Address for correspondence: Dr. Yogendr Bahuguna, Division of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun - 248 001, Uttarakhand, India. E-mail: yogendr.bahuguna@gmail.com
Received: 21-11-2008; **Accepted:** 07-02-2009**: DOI:** 10.4103/0973-8258.54910

regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water *ad libitum*. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study[4] was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD_{50}) was taken as an effective dose.[5]

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model[6] was used to assess the antilithiatic activity in albino rats. Animals were divided into seven groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II to VII for induction of renal calculi till 28th day. Group III received standard antiurolithiatic drug, cystone (750 mg/kg body weight) from 15th day till 28th day.[7] Groups IV and V served as curative regimen (CR). Group IV received aqueous extract (250 mg/kg body weight) and group V received alcohol extract (250 mg/kg body weight) from 15th day till 28th day, Group VI received aqueous extract (250 mg/kg body weight) and group VII received alcohol extract (250 mg/kg body weight) from 1st day till 28th day and served as preventive regimen(PR). All extracts were given once daily by oral route.

Assessment of Antiurolithiatic Activity
Collection and analysis of urine

All animals were kept in individual metabolic cages and urine samples of 24-h was collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium,[8] phosphate[9] and oxalate[10] content.

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 × g for 10 min and analyzed for creatinine, urea nitrogen[11] and uric acid.[12]

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2,000 × g for 10 min and the supernatant was separated.[13] The calcium[8] phosphate[9] and oxalate[10] content in kidney homogenate were determined.

Statistical Analysis

Results were expressed as mean ± S. D. Differences among data were determined using one-way ANOVA followed by Student Newman Keul's test (Graphpad Prism software for Windows, Version 4.10.1998). Differences between the data were considered significant at $P < 0.05$.[14]

RESULTS

From the acute toxicity study, the LD_{50} cut-off dose was found to be 2500 mg/kg body weight for both extracts. Hence, the therapeutic dose was taken as 250 mg/kg body weight for both extracts.

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals [Table 1, Group II]. However, supplementation with aqueous and alcohol extracts of *J. auriculatum* flowers significantly ($P < 0.001$) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney in curative regimens and preventive regimens as compared to cystone-treated animals [Table 1, Group III].

The deposition of the crystalline components in the renal tissues, namely oxalate, phosphate and calcium, was increased in the stone forming rats [Table 1, Group II]. The aqueous and alcohol extracts of *J. auriculatum* flowers treatment significantly ($P < 0.001$) reduced the renal content of these stone forming constituents in both regimens [Table 1, Group IV-VII].

Although the reduction was non-significant ($P > 0.05$) on inter-regimen comparison (curative regimens versus preventive regimens), the results were significantly ($P < 0.001$) comparable with cystone-treated [Table 1, Group III] animals.

The serum uric acid and blood urea nitrogen (BUN) were remarkably increased in calculi-induced animals [Table 1, Group II] while serum creatinine was only slightly elevated in Group II indicating marked renal damage. However, *J. auriculatum* flowers extract treatment in curative [Table 1, Group IV and V] and prophylactic [Table 1, Group VI and VII] regimen significantly ($P < 0.001$) lowered the elevated serum levels of creatinine, uric acid and BUN.

DISCUSSION

In the present study, male rats were selected to induce

Table 1: Effect of *Jasminum auriculatum* flower extracts on urinary and serum parameters in control and experimental animals

Parameter (unit)	Group I, normal (CI)	Group II, Calculi-induced (CT)	Group III, Cystone-treated	Curative regimen (CR I & CR II)		Preventive regimen (PR I & PR II)	
				Group IV, AlcE	Group V, AqE	Group VI, AlcE	Group VII, AqE
Urine (mg/dl)							
Oxalate	0.37 ± 0.03	3.64 ± 0.11*	0.53 ± 0.04***	0.87 ± 0.04***	1.28 ± 0.06**	0.78 ± 0.03***	1.07 ± 0.08**
Calcium	1.27 ± 0.07	4.51 ± 0.10*	1.50 ± 0.06***	1.76 ± 0.12***	1.90 ± 0.06**	1.67 ± 0.07***	1.86 ± 0.08**
Phosphate	3.64 ± 0.04	7.29 ± 0.06*	3.81 ± 0.09***	4.04 ± 0.08***	4.12 ± 0.10**	3.97 ± 0.06***	4.20 ± 0.09**
Kidney (mg/g)							
Oxalate	1.41 ± 0.06	5.73 ± 0.06*	1.61 ± 0.06***	1.83 ± 0.04***	2.12 ± 0.07**	1.75 ± 0.04***	2.06 ± 0.07**
Calcium	3.23 ± 0.04	4.79 ± 0.16*	3.42 ± 0.07***	3.68 ± 0.09***	4.10 ± 0.05**	3.58 ± 0.08***	3.96 ± 0.08**
Phosphate	2.35 ± 0.03	3.74 ± 0.10*	2.52 ± 0.07***	2.74 ± 0.08***	2.90 ± 0.10**	2.65 ± 0.07***	2.85 ± 0.07**
Serum (mg/dl)							
BUN	37.61 ± 0.15	49.97 ± 0.48*	39.30 ± 0.48***	40.80 ± 0.34***	42.87 ± 0.38**	40.35 ± 0.42***	41.11 ± 0.10**
Creatinine	0.75 ± 0.01	0.94 ± 0.03*	0.81 ± 0.02***	0.84 ± 0.01***	0.90 ± 0.01**	0.84 ± 0.01***	0.90 ± 0.02**
Uric acid	1.49 ± 0.07	3.64 ± 0.11*	1.71 ± 0.04***	1.88 ± 0.07***	2.11 ± 0.06**	1.87 ± 0.04***	2.05 ± 0.05**

Values are expressed as mean ± SEM of 6 observations; Statistical comparisons are made between Group N vs Group CI and CT (*P<0.001); Group CI vs PR I and PR II (**P<0.01); Group CT vs Group CR I and CR II (***P<0.01)

urolithiasis because the urinary system of male rats resembles that of humans[15] and earlier studies showed that the amount of stone deposition in female rats was significantly less.[16]

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidences in previous studies indicated that in response to 14 days period of ethylene glycol (0.75%, v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate[6,17,18]. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate.[17] Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate.[19,20]

In the present study, oxalate and calcium excretion are progressively increased in calculi induced animals (Group II). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria.[21] The changes in urinary oxalate levels are relatively much more important than those of calcium.[22] Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth.[23] However, aqueous and alcohol extracts of *J. auriculatum* flowers lower the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition.[24] Treatment of *J. auriculatum* flowers extract restores phosphate level, thus reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get accumulated in blood.[25] Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet.[26,27] In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane.[28] In calculi-induced rats (Group II), marked renal damage was seen by the elevated serum levels of creatinine and uric acid, and BUN. However, the curative and prophylactic treatment with aqueous and alcohol extracts of *J. auriculatum* flowers causes diuresis[29] and hastens the process of dissolving the preformed stones and prevention of new stone formation in urinary system.

CONCLUSION

The presented data indicate that administration of the aqueous and alcohol extracts of *J. auriculatum* flowers to rats with ethylene glycol induced lithiasis reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of *J. auriculatum*.

ACKNOWLEDGMENTS

The authors express their thanks to Botanical Survey of India, Dehradun, Uttarakhand, India for authentification of the plant

material. Authors also express their gratitude to Shri. Mahant Devendra Dass Ji Maharaj, Chairman, Shri. Guru Ram Rai Institute of Technology and Sciences, Dehradun, Uttarakhand, India for providing the facilities necessary to carry out the research work.

REFERENCES

1. Prien EL, Prien EL Jr. Composition and structure of urinary stone. Am J Med 1968;45:654-72.
2. Mukharjee T, Bhalla N, Aulakh GS, Jain HC. Herbal drugs for urinary stones - literature appraisal, Indian Drugs 1984;21:224-8.
3. Vaidyaratnam PS. Indian medicinal plants - a compendium of 500 species, Vol. 3., Hyderabad: Orient Longman Private Ltd ;2003. p. 164-5.
4. Ghosh MN. Fundamentals of experimental pharmacology. 2nd ed. Calcutta: Scientific Book Agency;1984. p. 156-7.
5. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from Andrographis paniculata against carbontetrachloride. Indian J Med Res 1990;92:276-83.
6. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniaria hirsuta on experimentally induced nephrolithiasis in rats. BJU Int 2003;92:137-40.
7. Mitra SK, Gopumadhavan S, Venkataranganna MV, Sundaram R. Effect of Cystone, a herbal formulation, on glycolic acid-induced urolithiasis. Phytother Res 1998;12:372-4.
8. Mustafa MA, Medeiros DM. Proximate composition, mineral content and fatty acids of cat fish (Ictalurus punctatus Rafinesque) for different seasons and cooking methods. J Food Sci 1985; 50:585- 8.
9. Fiske CH, Subbarow Y. The colorimetric determination of phosphate. J Biol Chem 1925;66:375-81.
10. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. Clin Chim Acta 1972;36:127-32.
11. Raghuramulu N, Madhavan NK, Kalyanasundaram S. A manual of laboratory techniques. 1st ed., Hyderabad: National Institute of Nutrition;1983. p. 34.
12. Caraway WT, Uric acid. In: Seligson D, editor. Standard methods in clinical chemistry., New York: Academic Press; 1963. p. 4239.
13. Chow FC, Dysart MI, Hamar DW, Udall RH. Control of oxalate urolithiasis by DL-alanine. Invest Urol 1975;13:113-6.
14. Kulkarni SK, Handbook of experimental pharmacology. 2nd ed. Mumbai: Vallabh Prakashan; 1993. p. 172-89.
15. Vermeulen CW. Experiments on causation of urinary calculi. Essays in experimental biology. Chicago: University of Chicago Press;1962. p. 253-69.
16. Prasad KV, Bharathi K, Srinivasan KK. Evaluation of (Musa parasidica Linn Cultivar)-"Puttubale" stem juice for antilithiatic activity in albino rats. Indian J Physiol Pharmacol 1993;37:337-41.
17. Selvam R, Kalaiselvi P, Govindaraj A, Bala Murugan V, Sathish Kumar AS. Effect of Aerva lanata flowers extract and Vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res 2001;43:89-93.
18. Huang HS, Ma MC, Chen J, Chen CF. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J Urol 2002;167:2584-93.
19. Selvam R, Adhirai M. Vitamin E pretreatment prevents cyclosporin A-induced crystal deposition in hyperoxaluric rats. Nephron 1997;75:77-81.
20. Muthukumar A, Selvam R. Effect of depletion of reduced glutathione and its supplementation by glutathione monoester on renal oxalate retention in hyperoxaluria. J Nutr Biochem 1997;8:445-50.
21. Tisselius HG. Solution chemistry of supersaturation. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM. editors. Kidney stones: Medical and surgical management. Philadelphia: Lippincott Reven;1996. p. 33.
22. Robertson WG, Peacock M. The cause of idiopathic calcium disease: hypercalciuria or hyperoxaluria? Nephron 1980;26:105-10.
23. Lemann J Jr, Worcestor EM, Gray RW. Hypercalciuria and stones. Am J Kidney Dis 1991;27:386-91.
24. Low RK, Stoller ML. Uric acid nephrolithiasis. Urol Clin North Am 1997;24:135-48.
25. Ghodkar PB. Chemical tests in kidney disease. Textbook of medical laboratory technology. Mumbai: Bhalani Publishing House;1994. p. 118-32.
26. Sumathi R, Jayanthi S, Kalpanadevi V, Varalakshmi P. Effect of DL- lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycollate treated rats. Pharmacol Res 1993;27:309-18.
27. Saravanan N, Senthil D, Varalakshmi P. Effect of L-cysteine on lipid peroxidation in experimental urolithiatic rats. Pharmacol Res 1995;32:165-9.
28. Ernster L, Nordenbrand K. Oxidation and phosphorylation. In: Ronald WE, Maynard EP, editors. Methods in enzymology. Vol. 10. New York: Academic Press; 1967. p. 574-80.
29. Yogendr B, Vijay J, Mohan Singh Maniyari R, Sunil J. Diuretic activity of flowers of *Jasminum auriculatum* Vahl. Journal of Pharmacy Research 2009;2:215-6.

Source of Support: Nil, **Conflict of Interest:** None declared.