Bulletin of Pharmaceutical Research 2013;3(1):40-3
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

EFFECT OF A POLYHERBAL FORMULATION ON GLYCOLIC ACID-INDUCED UROLITHIASIS IN RATS

Sarang Jain[1]* and Ameeta Argal[2]

[1]*Research Scholar, Institute of Pharmaceutical Science and Research Center, Bhagwant University, Sikar Road, Ajmer-305 004, Rajasthan, India*
[2]*Department of Pharmaceutical Chemistry, Rajeev Gandhi College of Pharmacy, Kolar Road, Bhopal-462 042, Madhya Pradesh, India*

**E-mails*: sarangjain123@rediffmail.com, ameetaargal@yahoo.com
Tel.: +91 9826418494, +91 9926905557.

Received: April 24, 2013 / Revised: April 29, 2013 / Accepted: April 30, 2013

The present study was done to evaluate the antiurolithiatic effect of a polyherbal formulation on glycolic acid-induced urolithiasis in rats. Oxalate urolithiasis was produced by the addition of 3% glycolic acid to the diet for a period for 42 days. In this study the level of oxalate, calcium and phosphorus was significantly increased whereas the level of sodium and potassium was significantly decreased. Treatment with cystone significantly decreases the level of oxalate, calcium and inorganic phosphorus. There was a significant increase in the kidney weight (both dry and wet weight) of animals receiving 3% glycolic acid which was significantly reduced by the treatment with cystone and polyherbal formulation. Results suggested that the increase in calcium and phosphate excretion could be due to defective tubular reabsorption in the kidneys while treatment with polyherbal formulation and ABP at the dose of 200 mg/kg markedly reduced the levels of these ions, showing the protective effect of polyherbal formulation and ABP (alcoholic *Bryophyllum pinnatum*) against urolithiasis.

Key words: Antiurolithiatic activity, Polyherbal formulation, ABP, Glycolic acid.

INTRODUCTION

Urinary stone disease continues to occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10%. Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia (Hiatt *et al* 1982).

The worldwide incidence of urolithiasis is quite high and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis (Anderson *et al* 1967; Robertson and Peacock, 1980). Recurrent stone formation is a common part of the medical care of patients with the stone disease. Calcium-containing stones, especially calcium oxalate monohydrate, calcium oxalate dehydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%.

In most of the cases the commonly occurring stones are calcium oxalate or magnesium ammonium phosphate type. Many remedies have been employed during the ages to treat urinary stones. In the traditional systems of medicine, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants (Coll *et al* 2002; Otnes, 1983; Williams, 1995). Keeping in view the

pharmacological properties of active ingredients of plants (Alam *et al* 2011; Dahiya and Gautam, 2011; Madaan *et al* 2011; Jain *et al* 2011; Zahid Hosen *et al* 2011; Chowdhury *et al* 2012; Dey *et al* 2012, Emran *et al* 2012), a polyherbal formulation containing alcoholic extracts *of Bryophyllum pinnatum* (Family - Crassulaceae, **Figure 1**), *Syzygium aromaticum* (Family - Myrtaceae, **Figure 2**) and *Ocimum sanctum* (Family - Lamiaceae, **Figure 3**) were prepared in the present study and the antiurolithiatic ability of ABP (Alcoholic *Bryophyllum pinnatum*) and polyherbal formulation was evaluated.

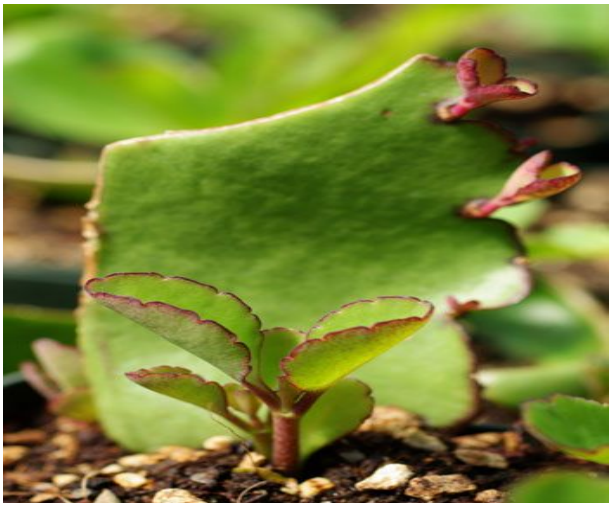

Fig. 1. Leaves of *Bryophyllum pinnatum*

Fig. 2. Buds of *Syzygium aromaticum*

MATERIAL AND METHODS

Plant material

The plant parts (leaves of *Bryophyllum pinnatum*, buds of *Syzygium aromaticum* and leaves of *Ocimum sanctum*) were procured from local market of Bhopal (MP) and authenticated from Department of Botany, Saifia College, Bhopal (Voucher No. 277/bio/saf/11/a, 278/bio/saf/ 11/b, 279/bio/saf/11/c).

Fig. 3. Leaves of *Ocimum sanctum*

Plant extraction

The plants were cleaned and chopped in to small pieces and dried under shade. The dried plant material was powdered and passed through the coarse sieve (No 10/44). This powder was macerated using alcohol and distilled water for 7 days with occasional shaking. The extract was filtered through muslin cloth, filtrate was evaporated under reduced pressure and vacuum dried. Each extract (0.714 mg) was taken and mixed to prepare a polyherbal formulation for assaying antiurolithiatic activity. (Khandelwal, 2003)

Animals

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 100 to 150 gm. They were provided from Sapience Bio-analytical Research Lab, Bhopal (MP). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C, relative humidity 44-56% and light and dark cycles of 12:12 h, fed with standard pallet diet and water ad libitum during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (Approval no. 1413/a/11/ CPCSEA).

Glycolic acid induced urolithiasis

The rats were divided into five groups of six each. Rats of group I received the commercial diet and served as control, group II was fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 42 days (Chow *et al* 1975). Groups III, IV and V received Cystone 750 mg/kg, alcoholic *Bryophyllum pinnatum* (ABP 200 mg/kg) and polyherbal formulation (200 mg/kg) respectively once a day orally in

addition to the CPD for 42 days. (Shah *et al* 2012; Mitra *et al* 1998).

Collection and analysis of urine samples

On day 42, immediately after administration of the respective assigned doses, the rats were housed in metabolic cages for 24 h urine collection. A drop of concentrated hydrochloric acid was added to the collected urine and stored at 4°C. Levels of oxalate (Hodgkinson *et al* 1972) calcium (Ohnishi, 1978) and inorganic phosphorus (Varley *et al* 1980) were determined spectrophotometrically. Sodium and potassium were estimated using a flame photometer.

RESULTS AND DISCUSSION

In the present study, urolithiasis was induced by the supplementation of normal commercial diet with glycolic acid for the 42 days. **Table 1** indicates the various serum mineral constituents - oxalate, calcium, phosphorus, sodium and potassium in control and experimental rats.

Table 1. Summary of effect of ABP and polyherbal formulation in glycolic acid induced urolithiasis model

Groups	Oxalate (mg/24h)	Calcium (mg/24h)	Inorganic Phosphorus (mg/24h)	Sodium (mEq/24h)	Potassium (mEq/24h)
Control	11.58±0.22**	4.72±0.21***	0.892±0.121**	11.28±0.45***	10. 21±0.79***
Negative control	23.21±2.10	8.11±0.52	1.421±0.161	5.31±0.82	6.21±0.62
Cystone (750 mg/kg)	12.2±2.52**	4.07±0.42***	0.802±0.121**	10.82±1.52***	10.89±2.32
ABP (200 mg/kg)	16.42±2.21	4.91±0.89	1.121±0.121	7.01±0.42*	7.84±1.61
Formulation (200 mg/kg)	13.50±2.12**	3.92±0.52***	0.921±0.152**	9.24±1.11***	9.70±1.61

Value represents, Mean±S.E.M. (n=6), Statistical analysis was performed by Dunnett's Multiple Comparison test, *p< 0.05, **p< 0.01, ***p< 0.001 as compared with group II

Calcium, phosphorus and oxalate play a vital role in renal calculogenesis (Richardson and Tolbert, 1961). In group II rats, level of oxalate, calcium and phosphorus was significantly increased whereas the level of sodium and potassium was significantly decreased. Treatment with cystone significantly decreases the level of oxalate, calcium and inorganic phosphorus. There was a significant increase in the kidney weight (both dry and wet weight) of animals receiving 3% glycolic acid which was significantly reduced by the treatment with cystone and polyherbal formulation. The increase in calcium and phosphate excretion could be due to defective tubular reabsorption in kidneys (Varalakshmi *et al* 1990) while treatment with polyherbal formulation and ABP at dose of 200 mg/kg markedly reduced levels of these ions, showing the protective effect of polyherbal formulation and ABP against urolithiasis (**Table 2**).

Table 2. Effect of ABP and Polyherbal formulation on kidney weight in glycolic acid induced urolithiasis model

Groups	Wet weight (g/100 g b.wt.)	Dry weight (g/100 g b.wt.)
Control	0.323±0.0041***	0.093±0.0016***
Negative Control	0.441±0.0068	0.121±0.0016
Cystone (750 mg/kg)	0.348±0.018***	0.095±0.0022***
ABP (200 mg/kg)	0.423±0.0028**	0.103±0.0034**
Formulation (200 mg/kg)	0.351±0.062***	0.097±0.0022***

Value represents, Mean ± S.E.M. (n=6), Statistical analysis was performed by Dunnett's Multiple Comparison test, *p< 0.05, **p< 0.01, ***p< 0.001 as compared with group II

The reduction in the urinary oxalate level will be beneficial in preventing urinary supersaturation with respect to oxalate. These results give a supportive evidence for the antiurolithiatic activity of ethanolic extract of ABP and polyherbal formulation.

CONCLUSION

Glycolic acid feeding for 42 days resulted in renal tissue deposition of calcium and oxalate. The increased deposition of calcium and oxalate in the renal tissue is known to lead to papillary calcification and eventual calculi formation. Polyherbal formulation and ABP administration significantly reduced both calcium and oxalate levels in the kidneys, which is known to prove beneficial in preventing calculi formation due to the supersaturation of these lithogenic substances. These effects suggest the antiurolithiatic property of ABP and polyherbal formulation.

REFERENCES

Alam K, Pathak D, Ansari SH. Evaluation of anti-inflammatory activity of *Ammomum subulatum* fruit extract. *Int. J. Pharm. Sci. Drug Res.* 2011;3(1):35-7.

Anderson EE, Rundles RW, Silberman HR, Metz EN. Allopurinol control of hyperuricosuria: A new concept in the prevention of uric acid stones. *J. Urol.* 1967;97(2): 344-7.

Chow FC, Dysart MI, Hamar DW, Udall RH. Control of oxalate urolithiasis by DL-alanine. *Invest. Urol.* 1975; 13(2):113-6.

Chowdhury S, Saha D, Paul S. *In vitro* cytotoxic activities of methanolic extract of *Mimosa pudica*. *Bull. Pharm. Res.* 2012;2(1):42-5.

Coll DM, Varanelli MJ, Smith RC. Relationship of spontaneous passage of ureteral calculi to stone size and location as revealed by unenhanced helical CT. *AJR Am. J. Roentgenol*. 2002;178(1):101-3.

Dahiya R, Gautam H. Solution phase synthesis and bioevaluation of cordyheptapeptide B. *Bull. Pharm. Res.* 2011;1(1):1-10.

Dey TK, Emran TB, Saha D, Rahman MA, Zahid Hosen SM, Chowdhury N. Antioxidant activity of ethanolic extract of *Cassia hirsuta* (L.) leaves. *Bull. Pharm. Res.* 2012;2(2): 78-82.

Emran TB, Rahman MA, Zahid Hosen SM, Saha D, Chowdhury S, Saha D, Dey TK. Antioxidant property of ethanolic extract of *Leucas aspera* Linn. *Bull. Pharm. Res.* 2012;2(1):46-9.

Hiatt RA, Friedman GD. The frequency of kidney and urinary tract diseases in a defined population. *Kidney Int*. 1982;22(1):63-8.

Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. *Clin. Chim. Acta* 1972; 36(1):127-32. [DOI: 10.1016/0009-8981(72)90167-2]

Jain RA, Agarwal RC, Pandey A, Jain R. Evaluation of *Argemone mexicana* fruits extract using micronucleus assay in mouse bone marrow cells. *Bull. Pharm. Res.* 2011;1(2):22-4.

Khandelwal KR. Practical Pharmacognosy, 12th edition, Nirali Prakashan: New Delhi, 2003; 149-51.

Madaan R, Bansal G, Sharma A. New phenolic glycosides from roots of *Actaea spicata* Linneaus. *Bull. Pharm. Res.* 2011;1(1):11-4.

Mitra SK, Gopumadhavan S, Venkataranganna MV, Sundaram R. Effect of cystone, a herbal formulation, on glycollic acid-induced urolithiasis in rats. *Phytother. Res.* 1998;12(5):372-4. [DOI: 10.1002/(SICI)1099-1573(199808)12:5<372::AID-PTR312>3.0.CO;2-X]

Ohnishi ST. Characterization of the murexide method: Dual-wave length spectrophotometry of cations under physiological conditions. *Anal. Biochem.* 1978;85(1):165-79. [DOI: 10.1016/0003-2697(78)90287-7]

Otnes B. Quantitative observations on the crystalline composition of urinary stones. *Scand. J. Urol. Nephrol.* 1983;17(2):185-90. [DOI: 10.3109/00365598309180166]

Richardson KE, Tolbert NE. Oxidation of glycolic acid to oxalic acid by glycolic acid oxidase. *J. Biol. Chem.* 1961; 236(5):1280-4.

Robertson WG, Peacock M. The course of idiopathic calcium stone disease: Hypercalciuria or hyperoxaluria?. *Nephron* 1980;26(3):105-10.

Shah JG, Patel BG, Patel SB, Patel R. Effect of *Hordeum vulgare* Linn. seeds on glycolic acid induced urolithiasis in rats. *Pharmacog. Commun.* 2012;2(2):34-9. [DOI:10.5530/pc.2012.2.5]

Varalakshmi P, Shamila Y, Latha E. Effect of Crataeva nurvala in experimental urolithiasis. *J. Ethnopharmacol.* 1990;28(3):313-21. [DOI: 10.1016/0378-8741(90)90082-5]

Varley H, Gowenlock AH, Bell M. Calcium, magnesium, phosphorus and phosphatase. *In* Practical Clinical Biochemistry, Vol II, 5th edition, The Whitefriars Press: London, 1980; 884-5.

Williams PL. The Anatomical Basis of Medicine and Surgery, 38th edition, ELBS & Churchill Livingstone: London, 1995; 1814-45

Zahid Hosen SM, Das R, Rahim ZB, Chowdhury N, Paul L, Saha D. Study of Analgesic activity of the methanolic extract of *Acorus calamus L.* and *Oroxylum indicum Vent* by acetic acid induced writhing method. *Bull. Pharm. Res.* 2011;1(3):63-7.

www.horizonherbs.com/images/products/x_bryophyllum_pinnatum.jpg

www.picsearch.com/Clove-pictures.html

http://2.imimg.com/data2/AJ/HT/MY-/tulsi-leaves.jpg

*****