



RESEARCH ARTICLE

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

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**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 decrease 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 (alcoholic *Bryophyllum pinnatum*) at the dose of 200 mg/kg markedly reduced the levels of these ions, showing the protective effect of polyherbal formulation and ABP against urolithiasis.**

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

## INTRODUCTION

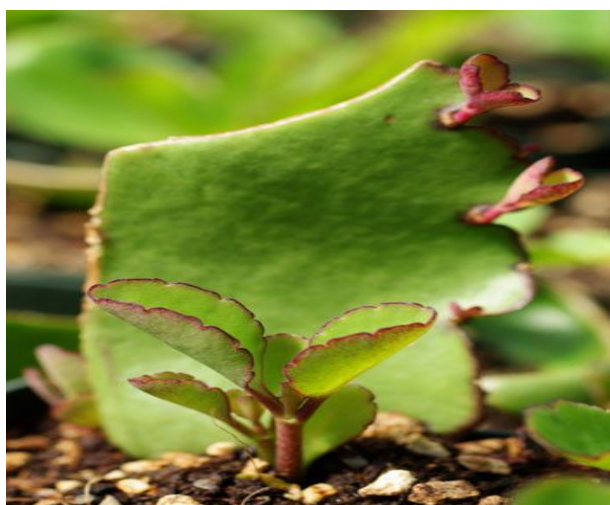
Urinary stone disease continues to occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10%. Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract 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  $25 \pm 2^\circ\text{C}$ , 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 the 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 42 days. **Table 1** indicates 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 would be beneficial in preventing the urinary supersaturation with respect to oxalate. These

results indicated 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 suggested the antiurolithiatic property of ABP and polyherbal formulation.

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