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](image1)

Fig. 1. Leaves of Bryophyllum pinnatum

![Fig. 2. Buds of Syzygium aromaticum](image2)

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](image3)

Fig. 3. Leaves of Ocimum sanctum

**Plant extraction**
The plants were cleaned and chopped into 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
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**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oxalate (mg/24h)</th>
<th>Calcium (mg/24h)</th>
<th>Inorganic Phosphorus (mg/24h)</th>
<th>Sodium (mEq/24h)</th>
<th>Potassium (mEq/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.58±0.22**</td>
<td>4.72±0.21***</td>
<td>0.892±0.121**</td>
<td>11.28±0.45***</td>
<td>10.21±0.79***</td>
</tr>
<tr>
<td>Negative control</td>
<td>23.21±2.10</td>
<td>8.11±0.52</td>
<td>1.421±0.161</td>
<td>5.31±0.82</td>
<td>6.21±0.62</td>
</tr>
<tr>
<td>Cystone (750 mg/kg)</td>
<td>12.2±2.52**</td>
<td>4.07±0.42***</td>
<td>0.802±0.121**</td>
<td>10.82±1.52***</td>
<td>10.89±2.32</td>
</tr>
<tr>
<td>ABP (200 mg/kg)</td>
<td>16.42±2.21</td>
<td>4.91±0.89</td>
<td>1.121±0.121</td>
<td>7.01±0.42*</td>
<td>7.84±1.61</td>
</tr>
<tr>
<td>Formulation (200 mg/kg)</td>
<td>13.50±2.12**</td>
<td>3.92±0.52***</td>
<td>0.921±0.152**</td>
<td>9.24±1.11***</td>
<td>9.70±1.61</td>
</tr>
</tbody>
</table>

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**

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

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


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


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