International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

EFFECT OF *BRYOPHYLUM CALYCINUM* SALISB ON EXPERIMENTALY INDUCED URINARY CALCULI

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ABSTRACT
Indian traditional system of medicine has contributed an important work in the field of plant activity. It claimed many of the plant activities which are considered as the best for treating disorders in world. Bryophylum calycinum Salisb is used for treating urinary disorders. Methanolic extract of dried leaves of Bryophylum calycinum was evaluated against 0.75% v/v ethylene glycol and 2% w/v ammonium chloride induced calcium oxalate urolithiasis in male albino rats. Treatment with the extract was able to reduce calculi formation, induced urinary excretion and renal deposition of calcium and oxalate, indicating its anticalculii effects. Results of the present study provide evidence for the claimed use of Bryophylum calycinum in the treatment of urinary disorders.

Key words: Bryophylum calycinum Salisb, Urinary calculi, Ethylene glycol, Ammonium chloride.

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INTRODUCTION

In spite of technological and conceptual developments in the present practice of medicine, the formation and growth of renal calculi continues to afflict mankind. The recognition of different types of urinary calculi resulted in more options of medical treatment[1]. Regardless of these advances, recurrence rates continue to be high and one of every two patients will develop other renal calculi within 5 years of the initial incident[2, 3] conforming a need to develop new drugs and treatments in order to prevent the recurrence of kidney stones. High urinary oxalate is a major risk factor for human idiopathic CaOx stone disease1. Studies indicate that oxidative stress mediates the pathogenesis of kidney stone disease[4, 5]. Though noninvasive and minimal invasive methods facilitated the treatment of kidney stone disease, recurrence is a common problem and makes it difficult and expensive to manage. *Bryophylum calycinum* is naturalized throughout the hot and moist part of India. The leaves and bark extract is

bitter tonic, astringent, carminative, analgesic, used in treatment of diarrhoea, vomiting[6] and antiulcer[7]. It also has anti-inflammatory[8, 9] and antimicrobial activity[10].

MATERIALS AND METHODS

Plant material

Fresh leaves of *Bryophylum calycinum* salisb were collected locally from Wagheshwar temple near Wagholi, Pune during July 2010 and authenticated by Dr.P.G.Diwakar, Joint Director, Botanical Survey of India (BSI), Pune, India. A voucher specimen of the plant was deposited in the JSPMs Charak College of Pharmacy and Research, Wagholi, Pune as an herbarium under the voucher number (TTSBC-1). The leaves washed thoroughly, dried in shade and coarsely powdered (40 mesh size). The methanolic extract of dried leaves was prepared using 70% (v/v) methanol by soxhlet method at a temperature of 60-70 ºC. The extract was then filtered, concentrated under vacuum and freeze-dried. A 10% w/v aqueous suspension was prepared from the concentrated extract just before dosing the rats. The extract was subjected to preliminary phytochemical testing.

Animal Selection

For acute toxicity studies, albino rats of either sex weighing between 150 and 200 g were selected for the Anticalculii activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 2 ºC) and maintained on 12 hr light: 12 hr dark cycle. They were housed in polypropylene cages and provided with regular rat chow and drinking water ad libitum. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) and were cleared by the same.

Acute Toxicity Studies:

The acute oral toxicity study (20) 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 (LD50) was taken as an effective dose[11, 12].

Assessment of anticalculii activity

Induction of calculii

The urinary calculii was induced by free access to drinking water containing 0.75% (v/v) ethylene glycol (EG) and 2% (w/v) ammonium chloride (AC) for15 days 9, 10.Four groups of rats consisting six per group were subjected to the following treatment[13,14].

Treatment schedule

Group I (**Normal):** Given distilled water (10 ml/kg, oral) from day 1-15 and given drinking water.

Group II (**Control):** Treated with distilled water (10 ml/kg, oral) and given drinking water containing EG and AC from day 1- 15.

Group III (MEBC **treated):** Received MEBC (125 mg/kg, oral) and given drinking water containing EG and AC from day 1-15.

Group IV (MEBC **treated):** Received MEBC (250 mg/kg, oral) and given drinking water containing EG and AC from day 1-15.

Anticalculii activity of the extract was studied by determining kidney weight, renal deposition and urinary excretion of calcium and oxalate and creatinine in all the groups of rats.

Urinary parameters

For group I-IV rats, 24 h urine samples were collected from overnight fasted rats after hydrating with distilled water (5 ml, oral) and placing them in separate metabolic cages on day 15. The supernatant obtained after centrifuging the samples at 2,500 rpm for 5 min was used to determine pH and quantitative estimation of calcium[11], oxalate[12] and creatinine[13].

Statistical analysis

Results were expressed as Mean ± SEM. One way analysis of variance (ANOVA) followed by Scheffes' test for multiple comparisons were used to measure intergroup variation. Statistical significance was considered at $p< 0.01$.

RESULTS

Preliminary phytochemical investigations

On preliminary phytochemical investigations using qualitative chemical tests, the methanolic extract

revealed the presence of Steroids, Alkaloids, Saponins, Carbohydrate, Amino acids, Polyphenols, Flavonoids, Tannins and Glycosides.

Acute toxicity and gross behavioral changes

Swiss albino male mice (weighing 20-25 g) and rats (weighing 150-200 g) were administered intraperitoneally with graded doses of methanolic extracts of the plant. After administration of the extracts the animals were observed for toxic effects during 24 hrs after the treatment. The toxicological effects were observed in terms of mortality and expressed as LD_{50}. The number of animals dying during the period was noted. The LD_{50} of the extracts were calculated by the method of Miller and Tainter.

Anti urinary calculi activity

A) Kidney weight

On administration of 0.75% (v/v) EG and 2% (w/v) AC in drinking water for 15 days, a significant increase in the kidney weight was observed in the control rats when compared to the normal rats. After 15 days of treatment with MEBC, a dose dependent significant reduction in kidney weight was observed in groups III and IV rats when compared to the control (Table 1).

Table.1 Effect of MEBC on Kidney weight, Oxalate and Calcium

Group	Treatment	Kidney weight (gm/100 gm body weight)	Oxalate (mg/gm wet kidney)	Calcium (mg/gm wet kidney)
I	Normal	0.34 ± 0.01	1.02 ±0.10	0.35 ±0.01
II	Control	0.50 ±0.02[a]	3.90 ± 0.21[a]	1.00 ±0.02[a]
III	MEBC (125 mg/kg)	0.39 ± 0.03[b]	2.65 ± 0.32[c]	0.55 ±0.03[c]
IV	MEBC (250 mg/kg)	0.35 ±0.01[c]	1.85 ± 0.23[c]	0.38 ±0.02[c]

Values are expressed as Mean± SEM

a- P < 0.001 compared to Normal

b- P < 0.01 compared to Control

c- P < 0.001 compared to Control

Deposition of calcium and oxalate in the kidney

On supplementation of MEBC, a dose dependent significant reduction in the EG and AC induced renal deposition of Calcium and Oxalate was observed in the groups III and IV rats (Table 1).

B) Urinary excretion of calcium, oxalate and creatinine

A dose dependent significant reduction in 24 hr urinary Oxalate, Calcium and Creatinine was observed in the MEBC treated rats (Table 2).

Table.2. Effect of MEBC on Oxalate, Calcium and Creatinine

Group	Treatment	Oxalate (mg/dl)	Calcium (mg/dl)	Creatinine (mg/dl)
I	Normal	1.98 ± 0.16	9.61 ± 0.21	8.62 ± 0.36
II	Control	10.11 ± 0.31[a]	24.12 ± 0.72[a]	30. 52 ± 0.38[a]
III	MEBC (125 mg/kg)	3.14 ± 0.19[b]	9.88 ± 0.13[b]	10.57 ± 0.68[b]
IV	MEBC (250 mg/kg)	1.78 ± 0.12[b]	10.06 ± 0.10[b]	8.31 ± 0.26[b]

Values are expressed as Mean ± SEM

a- P < 0.001 compared to Normal

b- P < 0.001 compared to Control

DISCUSSION

In the present study, urinary calculi were induced in rats by administrating ethylene glycol and ammonium chloride in drinking water orally for 15 days. Studies indicate that oral administration of EG which is a metabolic precursor of oxalate,

induces oxalate lithiasis in rats by being converted to endogenous oxalic acid in the liver[15], and AC when ingested, induces urinary acidification[16] thus favors adhesion and retention of calcium oxalate crystals within the renal tubules[17]. Super saturation of urine with calcium oxalate, the most common component of kidney calculi is an important factor in crystallization and enhanced urinary creatinine levels are indicators of renal impairment18. In the present study, on EG and AC administration in the control rats, enhanced excretion and deposition of calcium and oxalate indicate super saturation of urine with CaOx. Increased kidney weight also substantiated the results. In addition, enhanced urinary creatinine indicates hyperoxaluria promoted renal impairment. On administration of MEBC in the groups III and IV rats, the dose dependent reduction in urinary and renal calcium and oxalate compared to the control rats indicates MEBC prevented urinary supersaturation of calcium oxalate. Studies indicate that mucoproteins exhibit significant affinity for CaOx surface, thus promote the growth of crystals and cement them[19]. Saponins disintegrate the mucoprotiens there by prevent calcium oxalate excretion and deposition[20]. Saponins were reported to decrease CaOx crystal adhesion to renal epithelial cells by pre-coating the crystals. In the present study, the antilithiatic effect of MEBC may be attributed to its Saponin principles as the extract was found to contain saponins in preliminary phytochemical screening.

CONCLUSION

Above research showed that *Bryophylum calycinum* can be an alternative or an adjunctive measure to other therapies to prevent urinary calculi. The protective effects of MEBC against calculi formation may be attributed to its Saponin principles. However, further phytochemical and pharmacological investigations on *Bryophylum calycinum* are necessary to identify the antilithiatic chemical constituents that alleviate urinary calculi.

ACKNOWLEDGEMENTS

Authors are very much thankful to Jspms Charak College of Pharmacy and Research, Wagholi, Pune, for providing the facilities necessary to carry out the research work Authors also express their gratitude to Botanical Survey of India Pune, MS, India for authentification of the plant material.

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