Research Article

EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF HYDRO-ALCOHOLIC AND AQUEOUS LEAF EXTRACT OF *BARLERIA PRIONITIS* IN ALBINO RATS

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ABSTRACT

Leaves of *Barleria prionitis* (Family- Acanthaceae) are used in folklore medicine for various urinary ailments such as diuresis, urethral discharge and in dysuria. The present study is to investigate the Antiurolithiatic activity of Hydro-alcoholic and aqueous leaf extract of *Barleria prionitis* in Albino Rats. Renal calculi were induced in rats by supplementing water with 0.75 % v/v Ethylene glycol for 28 days. Antiurolithiatic activity was then measured by estimation of biochemical Serum (Blood Urea Nitrogen, Creatinine, Uric acid) and biochemical Urinary (Oxalate, Calcium, Phosphate) parameters. Preventive regimen (PR) and Curative regimen (CR) of *Barleria prionitis* Hydro-alcoholic extract (Alc.E) 400 mg/kg and Aqueous extract (Aq. E) 400 mg/kg significantly ($p < 0.001$) lowered the increased urinary and serum parameters whereas Preventive regimen and Curative regimen of *Barleria prionitis* Alc. E 200 mg/kg and Aq. E 200 mg/kg were less significant ($p < 0.05$). The histopathological evaluations of kidney samples were in support of the obtained result. Based on the above results a conclusion can be made that the *Barleria prionitis* Hydro-alcoholic and aqueous leaf extracts play a role in reduction and prevention of urinary stones.

Keywords: Antiurolithiatic, *Barleria prionitis*, Ethylene glycol, Hyperoxaluria, Cystone.

INTRODUCTION

Urolithiasis is the third most prevalent urinary disorder, about 80 % of which is composed of Calcium oxalate and Calcium phosphate^{1,2}. Hyperoxaluria is the promoting factor of Calcium oxalate stone disease. Oxalate causes crystallization in urine therefore its retention enhances cell injury and causes lithogenesis.³ Pathogenesis of urolithiasis is multi factorial, intricate and sex derived differences are thought to influence its incidence. Several previous reports have concluded testosterone to promote and estrogen to inhibit urolithiasis.⁴ Incidence of urinary tract stones is 2.4 times greater in men than in women, calcium oxalate composing about 74.9 % in males and 63.1 % in females. Management of urinary calculi depends on the size and the location of the calculi. Calculi that are larger than 5 mm or that fail to pass through urine are treated with surgical procedures such as extra corporeal shock wave lithotripsy (ESWL), Ureteroscopy (URS), or Percutaneous nephrolithotomy (PNL)⁵. Recurrence rate in urolithiasis is 50 % in 5 to 10 years, thereby causing economic consequences and being of great public health importance⁶. However, treatment with surgical procedures are costly, painful, require expertise and despite enjoying various advantages and numerous methods available for treatment by Allopathic system of medicine, it suffers from disadvantages that owe the patients to switch over to other forms of medicines like Ayurveda, Homeopathy and Unani. As Ayurveda is the most commonly used traditional system of medicine in India, traditional plants with less or no side effects are being documented for their scientific use. Thereby the present study is intended for the Evaluation of Antiurolithiatic activity of Hydro-alcoholic and aqueous leaf extract of *Barleria prionitis* in Albino rats.

MATERIAL AND METHODS

Chemicals

Cystone was procured from commercial source. Diagnostic kits for various biochemical analyses were procured from Galaxy Diagnostics Pvt. Ltd. Hyderabad, India.

Animals

Male Wistar albino rats weighing between 150 and 200 g were selected for acute toxicity studies and for the Antiurolithiatic activity. All the animals were housed in well ventilated cages at ($22 \pm 3^\circ\text{C}$) and maintained on 12:12 h light: dark cycle. They were fed with standard pellet and had free access to water. The animals were maintained in the above mentioned conditions a week prior the experimentation began. The experimental protocol described in present study was approved by Institutional Animal Ethical Committee (IAEC) with registration number of (1330/AC/10/CPCSEA).⁷

Plant Material

The leaves of *Barleria prionitis* were procured from Bhimavaram, West Godavari, Andhra Pradesh, India supplied by Ilas Challapatti during the month of March 2013. The collected plants were positively identified by Botanical Survey of India, Hyderabad, India with a Ref. No.BSI/DRC/2013-14/Tech./455.

Preparation of extracts

The aqueous extract (Aq E, 10 %, w/v) of *Barleria prionitis* leaves were prepared by maceration process (Chloroform-Water I.P), for 7 days at room temperature (yield 12.2 %, w/w) and the hydro-alcoholic extract (Alc E, 10 %, w/v) was prepared using 95 % (v/v) ethanol by soxhlation at 58°C (yield 23.5 %, w/w). The extracts were concentrated under vacuum. A suspension of Aq E and Alc E in 1 % CMC was prepared for oral administration by gastric intubation method. Alkaloids, flavonoids, saponins, tannins and Phenolic

compounds were revealed during phytochemical screening of the extracts.⁸

Experimental Design

Healthy male Wistar Albino rats (66) were divided in 11 groups containing six rats in each and kept in cages. All animals had free access to regular rat chow and drinking water *ad libitum* for 28 days⁹⁻¹¹.

Group I: Normal control rats will receive Normal saline p.o.

Group II: Negative Control (0.75 % v/v EG in drinking water)

Group III: Standard (0.75 % v/v EG +750 mg/kg Cystone; p.o)

Group IV (Preventive Regimen): Treatment (0.75 % v/v EG + 200 mg/kg *Barleria prionitis* (BP) Alc. E; p.o, 1 to 28 days)

Group V (PR): Treatment (0.75 % v/v EG + 400 mg/kg BP Alc. E; p.o, 1 to 28 days)

Group VI (PR): Treatment (0.75 % v/v EG + 200 mg/kg BP Aq. E; p.o, 1 to 28 days)

Group VII (PR): Treatment (0.75 % v/v EG + 400 mg/kg BP Aq. E; p.o, 1 to 28 days)

Group VIII (Curative Regimen): Treatment (0.75 % v/v EG + 200 mg/kg BP Alc. E; p.o, 15 to 28th day)

Group IX (CR): Treatment (0.75 % v/v EG + 400 mg/kg BP Alc. E; p.o, 15 to 28th day)

Group X (CR): Treatment (0.75 % v/v EG + 200 mg/kg BP Aq. E; p.o, 15 to 28th day)

Group XI (CR): Treatment (0.75 % v/v EG + 400 mg/kg BP Aq. E; p.o, 15 to 28th day)

Biochemical Analysis

Collection and analysis of urine

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. After urine collection, urine volume and pH of urine were measured. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate.¹¹

Serum analysis

After the experimental period, blood was collected from retro-orbital under anesthetic conditions and the animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 × g for 10 minutes and analyzed for Creatinine, Blood urea nitrogen (BUN) and Uric acid.¹¹

Histopathology

The abdomen was cut open to remove either kidney from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10 % neutral formalin. One of the isolated kidneys was then embedded in paraffin using conventional methods and cut into 5 µm thick sections and stained using hematoxylin–eosin dye and finally mounted in diphenyl xylene. Then the sections were observed under microscope for histopathological changes in kidney architecture and their photomicrographs were taken¹².

Statistical analysis

The data obtained by the various parameters was statistically evaluated by One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using Graph Pad Prism software (Graph Pad software Inc., Version 5.0). The mean values ± SEM were calculated for each parameter. Level of significance was kept at P < 0.05.⁷

RESULTS

Treatment of groups with EG 0.75 % showed increase of parameters in negative control group as compared to normal control group. Diuresis was significantly reduced and pH significantly increased in negative control group as compared to normal control group. Treatment with cystone (750 mg/kg; p.o.) and *Barleria prionitis*, Preventive and Curative regimen (Alc E – 200 mg/kg, 400 mg/kg; p.o. and Aq E- 200 mg/kg, 400 mg/kg; p.o) prevented the physiological changes induced by ethylene glycol. Various urolithiasis promoters (Calcium, Oxalate and Phosphate) level which enhances chances of stone formation was evaluated in urinary samples. Levels of these promoters were found to be increased in negative control as compared to normal control. This rise in the levels of promoters was significantly (p < 0.001) prevented by cystone (750 mg/kg; p.o.) and *Barleria prionitis* Preventive and Curative groups (p < 0001). Ethylene glycol induced negative control group showed marked increase in levels of BUN, Uric acid and Creatinine in serum as compared to normal control. Analysis of various biological samples revealed that treatment with standard drug cystone and *Barleria prionitis* alcoholic and aqueous extracts (Preventive and Curative regimen) significantly (p < 0.001) prevented the changes in level of BUN, Creatinine and Uric acid. No histopathological changes were found in normal treated group. The lithiatic group showed the presence of edema, inflammation and congestion. Treatment with Cystone (750 mg/kg b.w; p.o) significantly reduced the congestion. Histopathology of kidney samples treated with PR BP Alc. E 200 mg/kg showed little inflammation whereas PR BP Alc. E 400 mg/kg resembled like a normal kidney. PR BP Aq. E 400 mg/kg and EG 0.75 % treated groups showed mild congestion and mild inflammation. CR BP Alc. E 200 mg/kg showed inflammation and cyst deposition whereas CR BP Alc. E 400 mg/kg showed very few cysts. CR BP Alc. E 200 mg/kg showed medium crystal deposition while CR BP Alc. E 400 mg/kg showed very mild crystal deposition

DISCUSSION

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans. Earlier studies have also shown that the amount of stone deposition in female rats was significantly less than in male rats¹¹. Anti urolithiatic activity of Hydro Alcoholic leaf extract and aqueous leaf extract of *Barleria prionitis* was studied using Ethylene glycol induced urolithiasis model. 0.75 % v/v Ethylene glycol aqueous solution when administered to Wistar rats resulted in an increase in urinary oxalate levels. Calcium and Oxalate excretion in urine was increased in calculi-induced animals. Changes in renal tubular re absorption resulted in an increase of calcium deposition thereby leading to its urinary excretion. *Barleria prionitis* Hydro-alcoholic and aqueous leaf extracts reduced the levels of calcium and oxalate in urine thereby decreasing their retention in the kidneys¹³. Urinary phosphate levels were increased in EG treated groups. Urinary phosphate along with oxalate deposition enhances stone formation thereby causing calcium oxalate deposition¹⁴. *Barleria prionitis* extracts reduced the risk of calculi formation by decreasing the urinary phosphate levels. A significant increase in Urinary Serum parameters were reported in the study. The biochemical Serum parameters were decreased in the group of rats treated with the hydro-alcoholic and aqueous plant extract of *Barleria prionitis* in a dose dependent manner.

1. Normal Control

2. Negative Control

3. Positive Control

4. BP Alc.E 200mg/kg (PR)

5. BP Alc.E 400mg/kg (PR)

6. BP Aq.E 200mg/kg (PR)

7. BP Aq.E 400mg/kg (PR)

8. BP Alc.E 200mg/kg (CR)

9. BP Alc.E 400mg/kg (CR)

10. BP Aq.E 200mg/kg (CR)

11. BP Aq.E 400mg/kg (CR)

Figure 1: Photomicrographs of Kidney samples under 10X magnification

In urolithiasis, Oxalate by reacting with poly unsaturated fatty acids in cell membrane causes lipid peroxidation leading to renal tissue damage¹⁵⁻¹⁷. Elevated levels of Creatinine, Uric acid and BUN enhances renal damage. Maximum prevention of crystal deposition in histopathological evaluation was seen in preventive study which may be due to the presence of active compounds like Steroids, flavonoids and phenolic compounds in *Barleria prionitis* which have antioxidant property and which may prevent the calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

CONCLUSION

Administration of Ethylene glycol (0.75 % v/v) for 28 days resulted in the formation of calculi in the treated groups. Treatment with Hydro-alcoholic and aqueous extracts of *Barleria prionitis* significantly lowered the increased urolithiatic parameters when compared to calculi induced group. Based upon the above conclusion the Antiurolithiatic effect of *Barleria prionitis* may be attributed to its diuretic and anti-oxidant properties.

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Table 1: Effect of Hydro-alcoholic and Aqueous Leaf Extract of *Barleria prionitis* on Urinary Parameters

Parameters	Normal	Negative control	Positive control	Preventive Regimen				Curative Regimen			
				BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg	BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg
Urine Volume	12.15 $\pm$ 0.82	6.98 $\pm$ 0.72a	14.82 $\pm$ 0.36***	11.06 $\pm$ 0.36*	13.1 $\pm$ 0.30***	9.3 $\pm$ 0.41 ^{ns}	10.9 $\pm$ 0.70**	9.1 $\pm$ 0.46 ^{ns}	10.2 $\pm$ 0.50**	9.3 $\pm$ 0.73 ^{ns}	10.4 $\pm$ 0.32*
Urine pH	7.8 $\pm$ 0.05	6.2 $\pm$ 0.29a	7.5 $\pm$ 0.15***	7.1 $\pm$ 0.14 ^{ns}	7.6 $\pm$ 0.07***	7.0 $\pm$ 0.13 ^{ns}	7.3 $\pm$ 0.14*	7.14 $\pm$ 0.10 ^{ns}	7.52 $\pm$ 0.10**	7.32 $\pm$ 0.27 ^{ns}	7.41 $\pm$ 0.21*

All the values are Mean $\pm$ SEM, N = 6; a compared to normal group (p < 0.001), significances values are ***p < 0.001, **p < 0.01, *p < 0.05 (versus Negative control group)

Table 2: Effect of Hydro-alcoholic and Aqueous Leaf Extract of *Barleria prionitis* on Urinary Biochemical Parameters

Parameters	Normal	Negative control	Positive control	Preventive Regimen				Curative Regimen			
				BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg	BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg
Oxalate	11.44 $\pm$ 0.43	30.97 $\pm$ 0.323a	13.01 $\pm$ 0.20***	19.07 $\pm$ 0.207**	15.18 $\pm$ 0.146***	21.83 $\pm$ 0.20 ^{ns}	17.06 $\pm$ 0.13**	22.52 $\pm$ 0.211 ^{ns}	18.48 $\pm$ 0.23**	23.89 $\pm$ 0.254 ^{ns}	19.86 $\pm$ 0.12**
Calcium	4.40 $\pm$ 0.245	18.41 $\pm$ 0.43a	5.02 $\pm$ 0.077***	8.35 $\pm$ 0.15*	5.51 $\pm$ 0.33***	8.96 $\pm$ 0.18 ^{ns}	6.52 $\pm$ 0.108**	11.10 $\pm$ 0.155*	7.67 $\pm$ 0.09***	11.52 $\pm$ 0.07 ^{ns}	9.01 $\pm$ 0.085**
Phosphorous	2.96 $\pm$ 0.20	12.76 $\pm$ 0.42a	3.51 $\pm$ 0.109***	7.02 $\pm$ 1.06**	4.08 $\pm$ 0.29***	7.24 $\pm$ 0.083*	5.20 $\pm$ 0.26**	7.54 $\pm$ 0.073*	5.62 $\pm$ 0.19**	8.18 $\pm$ 0.09 ^{ns}	6.14 $\pm$ 0.087**

All the values are Mean $\pm$ SEM, N = 6; a compared to normal group (p < 0.001), significances values are ***p < 0.001, **p < 0.01, *p < 0.05 (versus Negative control group)

Table 3: Effect of Hydro-alcoholic and Aqueous Leaf Extract of *Barleria prionitis* on Serum Biochemical Parameters

Parameters	Normal	Negative control	Positive control	Preventive Regimen				Curative Regimen			
				BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg	BP Alc.E 200 mg/kg	BP Alc.E 400 mg/kg	BP Aq.E 200 mg/kg	BP Aq.E 400 mg/kg
BUN	31.36 $\pm$ 0.91	49.19 $\pm$ 0.20a	33.47 $\pm$ 0.350***	39.39 $\pm$ 0.103*	36.02 $\pm$ 0.437***	40.53 $\pm$ 0.191 ^{ns}	37.93 $\pm$ 0.415**	40.06 $\pm$ 0.12*	37.79 $\pm$ 0.142***	40.98 $\pm$ 0.207 ^{ns}	38.93 $\pm$ 0.25**
Creatinine	0.201 $\pm$ 0.09	1.332 $\pm$ 0.032a	0.301 $\pm$ 0.012***	0.675 $\pm$ 0.012**	0.411 $\pm$ 0.020***	0.733 $\pm$ 0.007 ^{ns}	0.560 $\pm$ 0.009**	0.772 $\pm$ 0.03*	0.524 $\pm$ 0.0078**	0.834 $\pm$ 0.01 ^{ns}	0.612 $\pm$ 0.011*
Uric Acid	0.43 $\pm$ 0.019	2.88 $\pm$ 0.037a	0.64 $\pm$ 0.024***	1.31 $\pm$ 0.014*	0.96 $\pm$ 0.023***	1.47 $\pm$ 0.025 ^{ns}	1.14 $\pm$ 0.035**	1.74 $\pm$ 0.021 ^{ns}	1.48 $\pm$ 0.024**	1.87 $\pm$ 0.02 ^{ns}	1.60 $\pm$ 0.037*

All the values are Mean $\pm$ SEM, N = 6; a compared to normal group (p < 0.001), significances values are ***p < 0.001, **p < 0.01, *p < 0.05 (versus Negative control group)