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EVALUATION OF DIURETIC AND ANTIUROLITHIATIC ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *BASELLA ALBA*

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ABSTRACT

The present study was undertaken to investigate the diuretic and antiurolithiatic activities of ethanolic leaf extract of *Basella alba* in Albino rats. Ethanolic leaf extract was administered to experimental rats orally at doses of 250mg/kg and 500mg/kg (each p.o). Furosemide (5mg/kg) was used as a standard. The diuretic effect of the extract was evaluated by measuring the urine volume and determining sodium, potassium, chloride and bicarbonate contents. Urolithiasis was induced in male rats by administering ethylene glycol (0.75%) in drinking water to groups II-V except normal control (Group I) for 28 days. Groups I, II and III served as normal control, positive control (hyperurolithiatic), and standard (cystone 750mg/kg), respectively, Groups IV and V served as curative regimen. Oxalate, calcium, phosphate were monitored in urine. Serum calcium, creatinine and uric acid were also recorded. The extract of *Basella alba* was safe and exhibited no gross behavioral changes in the rats. A significant diuretic effect was observed from the experimental animals treated with extract of *Basella alba* individually compared to the control. The results obtained suggest potential usefulness of extract of *Basella alba* leaf as an antiurolithiatic agent.

Key words: Diuretic activity, Natriuretic, Saluretic effect, Antiurolithiatic activity, *Basella alba*

INTRODUCTION

The drugs which increase the urinary output and electrolyte excretion are called diuretics. This drug mainly acts on the different parts of nephron and increase the urine out-put. Diuretic can also increase the excretion of electrolytes.[1] These drugs have more important effect in the treatment of oedema, acute and chronic renal failure, and moderate hypertension. High ceiling and osmotic diuretic agents are used in some poisoning condition to increase excretion of poisoning agent.[2] Urolithiasis refers to the solid non-metallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract.[3] It has been reported that 91% of the urinary calculi contain calcium in some form, while 8% and 1% are composed of uric acid and cystine [4] respectively. Several plants have been used to treat kidney stones including *Phyllanthus niruri, Agropyron repens* and *Herniaria hirsuta*.[5-7] *Basella alba* is known as Malabar spinach. The leaves are thick, rugose, succulent and green to purple colour.[8] Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, vitamins, alkaloids, quinines, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc.[9] *Basella alba* contains Vitamin A, Vitamin E, Vitamin K, flavonoids, saponins and β-Carotene. The plant is reported to treat against laxative, inflammation, rubefacient, skin diseases, burns, ulcers, diarrhoea, diuretic and cancer. The present study was under taken to evaluate invivo diuretic and antiurolithiatic activities of ethanolic leaf

extract of *Basella alba,* since no much reported work is published.

MATERIALS AND METHODS

Plant Materials: The leaves of Malabar spinach were collected from surrounding villages of Kakinada A.P. The plant was identified and authenticated by Dr. T.Raghuram, Taxonomist, Maharani college, Peddapuram.

Preparation of extract: The freshly collected leaves of the plant were cleared from dirt and dried under shade and then coarsely powdered manually. The leaf powdered was macerated in ethanol for a period of 7 days and later subjected to hot percolation for 8hrs. The extract obtained was subjected to solvent evaporation for complete drying.

Expermental Animals: Male albino rats weighing 150-200g were used for the study. The animals were housed in a temperature and light-controlled room (25^0c; 14h/10h light/dark cycle) with free access to food and drinking water. Prior to the animal studies the animals were acclamatized in the laboratory for a period of at least one week.

EXPERMENTAL DESIGN

DIURETIC ACTIVITY: Diuretic activity was determined following the Lipschitz method.[10] Rats weighing 150-200g were used for study. The rats [24] were fasted for 18hrs and deprived of water prior to the experiment. A priming dose of 25ml/kg of Normal saline was given to all rats. The rats were Grouped in to 4groups [6rats in each].

Group I: Control group and treated with vehicle, 0.5% acacia orally.
Group II: Treated with standard drug Furosemide [5mg/kg p.o] dissolved in vehicle.
Group III & IV: Were treated with ethanolic leaf extract of *Basella alba* [250mg/kg & 500mg/kg p.o] respectively.

Immediately after the administration the rats were placed in metabolic cages, one rat per cage. The metabolic cages provided with a funnel for urine collection and a mesh to separate the faeces from the urine. The bladder was emptied by pulling the base of the tail of each rat.[14] The volume of urine collected was recored after 5hrs and urine was subjected to analysis for sodium, potassium ions by Flame photometry,[15] chloride and bicarbonates by titrimetric analysis[16] after 24 hrs. The Saluretic, Natriuretic and Diuretic Indices were also calculated.

***Analysis of urine*:** The urine collected into beaker was covered with aluminum foils to avoid evaporation. Analysis of the urine included estimation of sodium, potassium, chloride and bicarbonate ions.

ANTI UROLITHIATIC ACTIVITY: Ethylene glycol-induced hyperoxaluria method [11] was used to assess the antiurolithiatic activity in albino rats. Animals were divided into five groups containing six animals in each group. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of renal calculi for 28days. Group II served as calculi induced (hyperurolithiatic). Group III received standard antiurolithiatic drug, cystone (750mg/kg b.w.), from15^{th} day till 28^{th} day.[12] Group IV and V served as curative regimen (CR). Group IV received Ethanolic extract (250mg/kg b.w.) and Group V received (500mg/kg b.w.) from 15^{th} day till 28^{th} day. All extracts were given once daily p.o.

Assesment of Antiurolithiatic activity:

Collection and analysis of urine: Rats were kept separately in metabolic cages and urine samples of 24h were collected on 28^{th} day. A drop of conc.hydrochloric acid was added to the urine before being stored at 4^0c. Urine samples were analyzed for calcium, phosphate and oxalate content.

Serum analysis: At the end of experiment, blood samples were collected from the tail-vein and analyzed for creatinine, calcium, and uric acid.[13] The samples were analyzed using Biochemistry analyzer.

Histopathological studies: The animals were anesthetized with diethylether on 29^{th} day, and the abdomen was cut opened and kidneys were removed. The kidneys were stored in formalin(10%), fixed and soaked in paraffin, cut at 2-3µm thin, and the slices were stained using hematoxylin and eosin. Tissue slices were photographed using optical microscopy under polarized light.

Statistical analysis: The results were expressed as the mean ± SEM and analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and $P< 0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Acute toxicity studies observed that animals tolerated a maximum dose of 2000 mg/kg b.w. with no noticeable behavioral changes in all groups. *Basella alba* showed significant (P<0.001) diuretic effect when compared to the control group. The test drug increased the urine volume and significantly enhanced urinary Na^+, K^+, Cl^- and Hco_3^- excretion as shown in (**Table I**). Diuretic, Saluretic, Natriuretic Indices of ethanolic leaf extract of *Basella alba* was shown in (**Table II**). Hyperoxaluria induced by chronic administration of 0.75 %(v/v) ethylene glycol increased oxalate, calcium and phosphate excrection (GroupII). Ethanolic leaf extract of *Basella alba* significantly (P<0.001) lowered the elevated levels of oxalate, calcium and phosphate in urine (CR) as compared to calculi-induced animals (**Table III**). The deposition of the crystalline components in the renal tissue namely oxalate, calcium and phosphate, were increased in the stone forming rats (Group II). The Ethanolic leaf extract of *Basella alba* treatment significantly (P< 0.001) reduced the renal content of stone forming constituents in GroupV. The present study revealed that apart from renal protection, the ethanolic extract of *Basella alba* possessed significant diuretic activity. A significant increase in the sodium ions in the urine supports that the extract can be used to treat hypertension. The possible mechanism involved might be an increase in Glomerular filtration rate and decreased tubular reabsorption. Chronic administration of 0.75% ethylene glycol aqueous solution to male rats resulted in hyperoxaluria. Urinary stone formation is result of super saturation of urine with certain urinary salts such as calcium oxalate. Ethanolic leaf extract of *Basella alba* significantly lowered the levels of oxalate, phosphate and calcium in urine and calcium, creatinine and uric acid in serum. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction of the out flow of urine by stones in urinary system. Due to this, the waste products such as urea, creatinine, and uric acid get accumulated in blood. In calculi-induced rats, the elevated serum levels of creatinine, uric acid, and calcium indicate marked renal damage. Renal histological analysis stained with hematoxylin and eosin featured the following observations. In **Figure I**: Group I Kidney of control rats showed normal features with prominent cortical tubules and Bowman's capusle. Group II: Kidney of urolithiatic treated (0.75%EG) rats showed distorted renal tubules, marked inflammation & hemolysis. Group III: Kidney of cystone (750mg/kg) rats showed morphology as compared to signs of inflammation reduced, there was no hemolysis. Size of glomerulus was normal. Group V: Kidney of ethanolic Ext (500mg/kg) treated rats have shown minimal signs of inflammation & hemolysis.

CONCULSION

From the experimental results and evidence it can be concluded that the Ethanolic leaf extract of *Basella alba* leaves possess significant diuretic activity by increasing the total urine output and increased excretion of sodium, potassium, chloride and bicarbonate levels, and also possess significant antiurolithiatic activity by lowering the elevated levels of oxalate, calcium and phosphate in urine, further calcium, creatinine, and uric acid in serum. Hence, isolation of active principles will be advantageous to produce novel bioactive constituents from the extract which may possess more significant biological activities.

Table I : Comparison of Diuretic effect of *Basella alba* to that of control

Group	Volume of urine(ml) After 5h	Na^+ µmole/kg	K^+ µmoles/kg	Cl^- µmoles/kg	HCO_3^- µmoles/kg
Group I control	0.15 ±0.04	173.33 ± 0.35	121.48 ±0.48	98.66 ±0.59	09.97 ±0.17
Group II standard Furosemide	0.74 ±0.01**	232.14 ±0.65**	144.34 ±0.20*	152.52 ±0.39**	25.36 ±0.33**
Group III (B.a 250mg/kg)	0.31 ±0.03	180.23 ±0.03	124.93 ±0.45	140.98 ±0.60*	24.24 ±0.53*
Group IV (B.a 500mg/kg)	0.52 ± 0.06*	196.21 ±0.52	128.76 ± 0.53	158.92 ± 0.39***	27.10 ±0.39**

Values are expressed as mean ± SEM, n=6, Comparisions are made with Group I; P<0.001

Table:II Comparison of Saluretic, Natriuretic and Diuretic Index of extract to that of control.

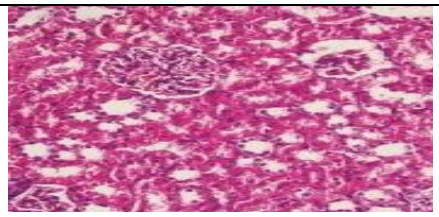
Group	Saluretic	Natriuretic	Diuretic
Group I control	272.0	1.42	_
Group II standard	384.6	1.61	4.9
Group III (B.a	321.2	1.45	2.0
Group IV (B.a	355.1	1.53	3.4

Diuretic index = volume of urine in test group/volume of urine in control group

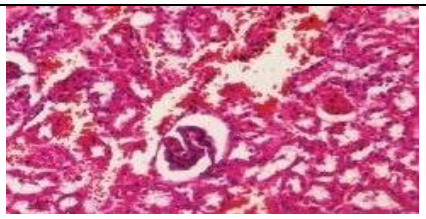
Table:III Effects of extract of *Basella alba* leaf on oxalate, calcium, phosphate in rats

Parameter (unit)	Group I control	Group II, calculi induced	Group III, cystone treated (750mg/kg)	Curative regimen (250mg/kg) Group IV	(500mg/kg) Group V
Urine (mg/dL)					
Oxalate	0.61 ± 0.14	3.16 ± 0.13*	0.59 ± 0.03	2.78 ± 0.04*	0.98 ± 0.20*
Calcium	1.09 ± 0.08	3.77 ±0.18*	1.58 ± 0.04*	3.70 ± 0.36*	2.9 ± 0.30*
Phosphate	3.32 ± 0.21	6.91 ± 0.21*	3.69 ± 0.14*	5.92 ± 0.28*	4.28 ± 0.21*
Serum (mg/dL)					
Calcium	7.98 ± 0.53	14.38 ± 0.45*	7.09 ± 0.24*	11.92 ± 0.62**	8.5 ± 0.32*
Creatinine	0.71 ± 0.02	1.01 ± 0.04*	0.78 ± 0.26	0.99 ± 0.11*	0.72 ± 0.50
Uric acid	3.917 ± 0.25	6.13 ± 0.20*	4.38 ± 0.19*	4.78 ± 0.27*	3.2 ± 0.17

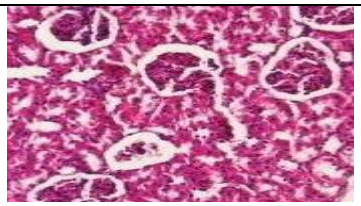
Values are expressed as mean ± SEM, n=6, Comparisions are made with Group I; P<0.001.



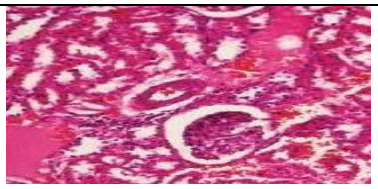
Group I: Kidney section of control rats 40x



Group II: Kidney section of urolithiatic rat (EG 0.75%) 40x



Group III: Kidney section of Cystone (750mg/kg)treated rat 40x



Group V : Kidney section of Plant Ext (500mg/kg) treated rat 40x

Figure I: HISTOPATHOLOGY STUDIES

Histology of kidney sections. There is marked inflammation & hemolysis in Group II rats. Histology of Group III showed restored morphology and Signs of inflammation reduced, there was no hemolysis and Group V shows minimal signs of inflammation & hemolysis.

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