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Phytochemical and Diuretic Activity of *Barleria Strigosa* Leaves

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Abstract
The present study was carried out to evaluate the diuretic effect of *Barleria prionitis* Linn. flower extract in rats. Diuretic and Natriuretic activities were carried out by administration of normal saline along with the treatment modules. The volume of urine (in ml) and the Na+ and K+ content in the urine were measured. The extract at 100 and 200 mg / kg, produced significant diuresis and increased sodium elimination but not potassium. Thus the study elucidates that aqueous extract of *Barleria prionitis* posess significant diuretic and Natriuretic effect but not a potassium sparing effect.

Keywords: *Barleria prionitis*, Diuresis, Natriuretic, Sodium, Potassium

Introduction
Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs [1]. The study of plant species with diuretic effects is still a fruitful research in search of new diuretics. Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and an accompanying anion, usually Cl^-. Most clinical applications of diuretics aim to reduce extracellular fluid volume (edema) by decreasing total body Nacl content. Although continued administration of diuretic causes a sustained net deficit in total Na^+, the time course of natriuresis is finite because renal compensatory mechanisms brings Na^+ excretion in line with the Na^+ intake, a phenomenon known as diuretic braking. Diuretics alter the excretion of other cations (e.g. K^+, H^+, Ca^{2+}, Mg^{2+}), anions (e.g. Cl^-, HCO_3^- and H_2PO_4) and uric acid. In addition diuretics may alter renal hemodynamics indirectly mediated by local prostaglandins synthesis [2].
Barleria strigoes L. (Family Acanthaceae; commonly known as Bristly Blue Barleria), is an upright shrub, 0.8 m high, it occurs naturally in the foothills of the Himalayas, but has been introduced to far northern Queensland, Australia.. Traditionally, *B. strigosa* is used for treating fever, flu, nose bleeding and as an antidote for detoxification of poisons [3, 4]. The important phytochemical compounds isolated from Barleria are iridoids, phenolic acids, phenylethanoid glycosides lignans, flavonoids, and phytosterols [5]. Despite the popular use of this species as a medicinal plant, there are no data about the pharmacological effect of leavesof B. strigose on diuretic activity. The aim of the present study was to evaluate the potential diuretic activities *Barleria Strigosa* leaves extract on different experimental animal.

Materials and methods
Collection and Authentication of plant material
The plant specimen was collected from natural habitat of Ranga Reddy District, Telangana, India. The taxonomic identification of the plant was confirmed by Dr. Madhavan Chetty, Assistant Professor, Sri Venkateswar University, Tirupathi, Andhra Pradesh. The plant material was dried in sunlight, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

Preparation of extract
The freshly collected Barleria. stigose leaves were washed with distilled water and air-dried under the control conditions and powdered. The powder was subjected to defat with petroleumether and subjected to maceration with methanol. The methanol extract was dried and weighed. (yield 14.10 % w/w).

Experimental animals
Healthy male albino rats weighing 180-200 g were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 37 ± 1°C and standard 12h : 12h day/night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd.) and water ad libitum. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Drug Treatment
The BSL extract (suspended in 1% carboxy methyl cellulose) at the dose levels of 250, 375 and 500 mg/Kg body wt., p.o. was administered once daily for three consecutive days. Furosemide (20 mg/Kg; p.o.) was used as standard for diuretic activity. Control group of animals (n=6) received suspension of 1% CMC in distilled water(10 ml/Kg).

Experimental design
Thee animals were divided into 5 groups of 6 rats each as follows; Group I: received only 1% g CMC Group II: received MEBSL 250 mg/kg, Group III: received MEBSL 375 mg/kg body weight p.o., Group III: received MEBSL 500 mg/kg body weight p.o., Group V Furosemide 20 mg/kg.

In-Vivo Evaluation of diuretic activity (Lipschitz model):
Thirty rats were deprived of water but not food for 18 hours. Their bladders were emptied by pulling on their tails and gently compressing the pelvic region. To impose a homogeneous water load, each of these rats was given 15 ml of isotonic saline (NaCl, 0.9 % w/v) orally. After 45 minutes, the rats were randomly divided into four groups (n = 6 per group) and given the following oral treatment. One ml of pure water, 250 mg/kg QL of extract, 375 mg/kg of QL extract, and 500 mg/kg of QL extract were used in the first three groups [6], while Group 5 received 13 mg/kg of Furosemide [7] as standard.
For adaptation, each animal was isolated in metabolic cages for 24 hours prior to the start of the experiment, and then starved overnight with free access to water. Urine samples were taken at 6 hours, 12 hours, and 24 hours following the previous dosage. For electrolyte analysis, the urine samples were filtered and then kept at -20°C.

Urine Parameters Measurements
For all rats, cumulative urine output was assessed after 6, 12, and 24 hours. A digital pH meter and a conductivity meter were used to test the pH and conductivity of fresh urine samples. The colour of urine was also noted. The entire urine output samples (24 hours) were then diluted (1:1000 in deionized water) to measure the total electrolytes (sodium, potassium, and chloride ions) concentrations in urine using a Flame Photometer [8].

Kidney Homogenate Preparation
At the termination of the experiment, the animals were decapitated and slaughtered. Arteovenous blood was drawn and centrifuged in heparinized tubes. The obtained plasma was maintained at 20°C for biochemical examination. The kidney was removed, defatted, weighed, and kept at 20°C for further biochemical examination.

Serum and Homogenate Parameters Measurements
A Double Beam Spectrophotometer (Shimadzo) was used to measure the concentrations of creatinine, urea, aldosterone, glucose, albumin, and electrolytes in plasma and urine samples. In addition, urinary osmolality and natriuretic were assessed in animals treated with the extract and reference compounds during the diuretic response, notably at the highest excretion rate. An osmometer (YK Analytical Corporation, Pune) was used to test the osmolarity of plasma and urine samples. Aldosterone radioimmunoassay was used to determine aldosterone levels by Aldosterone RIA (CT). Osmolar clearance (C_{osm}) was calculated from plasma osmolality (P_{OSM}), urinary osmololarity (U_{osm}), and urine flow (V) using the formula:

$$C_{osm} = U_{osm} \times V/P_{OSM}.$$

When solutes are eliminated in more water than filtered plasma volume water, free water clearance ($C_{H2O} = V\text{-}C_{osm}$) is positive. The clearance of creatinine determines glomerular filtration. Creatinine clearance was used to calculate GFR (glomerular filtration rate) ($C_{reat}C$). The quantity of Na^+ and K^+ was computed as a saluretic activity parameter. The natriuretic activity was determined using the Na^+/K^+ ratio. The $Cl/(Na^+ + K^+)$ ratio was used to assess the inhibitory activity of carbonic anhydrase [9].
Each of these rats was individually placed in metabolic cages and cumulative urine output will be determined at hourly intervals for 5 h. The colour of urine will also note. In an attempt to ascertain the broad mechanisms of action, the urine collected from group 1 (control) and group 4 will be subjected to the following investigations: pH by pH meter, Na+ and K+ levels by flame photometry, osmolality by Osmometer, glucose and proteins (Reagent strips) [7].

Statistical evaluation
All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett's t-test using GraphPad Prism 9. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$.

Result and Discussion
Effect on Urine Output and Diuretic Activity
Table 1specify the urine volume, diuretic effect, and diuretic activity. The total urine volume was assessed for the *Barleria Strigosa* leaves methanolic extracts (250, 375, and 500 mg/kg), standard diuretic (furosemide), and normal control during periods of 6, 12, and 24 hours. According to the findings, *Barleria Strigosa* leaves displayed diuretic action at all doses tested, including furosemide at 6, 12, and 24 hours, and the impact was dose dependent. When compared to control rats, furosemide and *Barleria Strigosa* leaves substantially enhanced urine flow at 6, 12, and 24 hours ($p< 0.001$). The high dosage voided more than twice as much urine as that of control.

Table 1: Effect of methanolic extract of *Barleria Strigosa* leaves on volume of cumulative urinary excretion after 6, 12 and 24 hrs

Groups	After 6 hrs			After 12 hrs			After 24 hrs		
	Urine Volume (ml)	Diuretic action [a]	Diuretic activity [b]	Urine Volume (ml)	Diuretic action [a]	Diuretic activity [b]	Urine Volume (ml)	Diuretic action [a]	Diuretic activity [b]
Control	80 ± 1.3	1	0.544	165 ± 2.4	1	0.57	244 ± 4.9	1	0.55
Extract 250mg/kg	95 ±1.1 ***	1.19	0.646	187 ± 2.2***	1.13	0.65	284 ± 4.1***	1.16393	0.64
Extract 375mg/kg	102 ±0.9 ***	1.27	0.694	210 ± 1.53***	1.27	0.73	318 ± 3.96***	1.30328	0.72
Extract 500mg/kg	124 ± 0.7***	1.55	0.844	248 ± 1.21***	1.5	0.86	388 ± 2.63***	1.59016	0.88
Furosemide 13mg/kg	147 ± 0.8***	1.84	1.000	288 ± 1.47***	1.75	1.00	443 ± 2.79***	1.81557	1.00

All Values are expressed as mean±SEM where n=6.
[a] Diuretic action=urine volume of test/urine volume of control.
[b] Diuretic activity=urine volume of test /urine volume of Standard (Furosemide).
***Significant difference at p< 0.001 as compared with control group.

Effects on urine pH and conductivity

The pH and conductivity of urine were tested after 24 hours. Control rats had a urine pH of 6.11±0.02. At 24 hours following administration of *Barleria Strigosa* leaves extract at dosages of 250, 375, and 500 mg/kg body weight, the urine pH was 6.73±0.13, 7.25±0.09, and 7.89±0.16 respectively. Furosemide made the urine significantly alkaline by raising the pH to 8.08±0.21 (P<0.001). At 24 hours, the conductivity of control rats was observed to be 12.57±0.31. The urine conductivity of furosemide-treated rats increased considerably to 19.27±0.19. The urine conductivity of rats treated with *Barleria Strigosa* leaves extract was 14.32±1.09, 17.44±0.89, and 18.87± 0.65 at dosages of 250, 375, and 500 mg/kg, respectively.

Effects on electrolyte excretion

As compared to normal control rats, the diuretic responses of the methanolic *Barleria Strigosa* leaves extract with its electrolyte excretion potency were very moderate at all dosages. The *Barleria Strigosa* leaves extract at doses of 375 and 500 mg/kg showed a significant increase in Na+, K+, and Cl- excretion. The results of urinary electrolyte excretion after treatment of *Barleria Strigosa* leaves extract were comparable to the furosemide group also (Table 5.4). The effects of methanolic extract of *Barleria Strigosa* leaves on Na+, K+, and Cl- excretion at 6, 12, and 24 hrs are shown in Table 2.

Table 2: Effect of methanolic extract of *Barleria Strigosa* leaves on electrolyte excretion after 24 hrs

Groups	Urinary Na+		Urinary K+		Urinary Cl-	
	Na^+ excretion[a] (mEq/kg/24hrs)	Na^+ Index[b]	K^+ excretion[a] (mEq/kg/24hrs)	K^+Index[b]	Cl^- excretion[a] (mEq/kg/24hrs)	Cl^-Index[b]
Control	18±1.00***	1	19±0.82***	1	15±2.10***	1.0
Extract 250mg/kg	52 ± 2.12***	2.9	37±1.41***	1.9	50±2.13***	3.3
Extract 375mg/kg	102±1.25***	5.7	100±0.71***	5.3	100±1.03***	6.7
Extract 500mg/kg	150±1.78***	8.3	47±2.35***	2.5	150±1.42***	10.0
Furosemide 13mg/kg	92±5.96***	5.1	34±2.62***	1.8	90±6.21***	6.0

[a]All Values are expressed as mean±SEM where n=6.
[b] Index=Excetion of test/excretion of control.
[b] Diuretic activity=urine volume of test /urine volume of Standard (Furosemide).
***Significant difference at P< 0.001 as compared with control group.

Effects on natriuretic, saliuretic and carbonic anhydrase inhibition

Table 3 depicts the results for natriuretic, saliuretic, and carbonic anhydrase inhibition. The furosemide (13 mg/ kg) and methanolic extract of *Barleria Strigosa* leaves at doses (375 and 500 mg/kg) showed potent natriuretic and saliuretic activity as compared to normal control. In our study, the methanolic extract of *Barleria Strigosa* leaves showed less effect on carbonic anhydrase inhibition.

Table 3: Effect of methanolic extract of *Barleria Strigosa* leaves on Saluretic, Natriuretic and carbonic anhydrase inhibition after 24 hrs

Groups	Saluretic Effect (Na^++ Cl^-)[a]	Natriuretic Effect (Na^+/K^+)[a]	CAI Cl/(Na^++ K^+)[a]	Saluretic index[b]	Natriuretic index[b]	CAI index[b]
Control	33.2 ± 3.1	0.9±1.8	0.4± 0.65*	1.0	1.0	1.0
250 mg/kg	101.9± 4.18***	1.4± 1.30**	0.6± 0.4*	3.1	1.5	1.4
375 mg/kg	202.5± 2.19***	1.9± 1.65**	0.6± 0.33*	6.1	2.0	1.6
500 mg/kg	300.1± 3.32***	3.3± 70***	0.8± 0.32**	9.0	3.5	1.9
Furosemide (13mg/kg)	182.8± 12.57***	2.6± 2.35***	0.7± 0.39**	5.5	2.8	1.8

[a]All Values are expressed as mean±SEM where n=6.
[b] Index=Effect of test/excretion of control. CAI: Carbonic Anhydrase Inhibition
*** p< 0.001, ** p< 0.01, *p< 0.05, Significant difference as compared to the control

Effects of *Barleria Strigosa* leaves on Serum Parameters

Some hematological parameters were evaluated in rats treated with the plant extract and pharmacological substances used as a check. The glucose concentration of rats ranged between 93.28±10.35 mg/dl and 97.09 ± 6.12 mg/dl in animals in disregard of the treatment. There was a significant increase ($P < 0.01$) of creatinine and urea in animals that received the extract at dose 500 mg/kg. The animals receiving Furosemide also showed a significant increase ($P < 0.01$) in the rate of creatinine and urea. Albumin increased from 41.4 ± 4.89 g/l in the control group to 44.2 ± 4.99g/l and 44.4 ± 6.0g/l in animals that received the extract at a dose of

375mg/kg and 500mg/kg respectively and with Furosemide, there was a significant increase ($P < 0.01$) in the albumin concentration to 43.1 ± 5.05. The concentrations of Na+ and K+ ions were significantly increased ($P < 0.01$), in animals that received the extract at the dose of 375mg/kg and 500mg/kg respectively (Table 4). There was a significant increase ($P < 0.01$) in aldosterone and plasma osmolality levels in animals treated with extract at 375mg/kg, 500mg/kg and Furosemide.

Table 4: Effect of methanolic extract of *Barleria Strigosa* leaves seeds on serum parameters

Parameters	Groups				
	Control	Extract 250 mg/kg	Extract 375 mg/kg	Extract 500 mg/kg	Furosemide (13 mg/kg)
Glucose (mg/dl)	93.28±10.35	96.25 ±10.25*	95.22±11.20*	96.24 ± 6.95*	97.09 ± 6.12*
Creatinine (mg/dl)	0.59 ± 0.40	0.66 ± 0.70*	0.69 ± 0.44*	0.85 ± 0.18**	0.74 ± 0.19**
Urea (mg/dl)	22.16 ± 4.15	23.38 ± 2.14*	23.44 ± 4.66*	24.86 ± 5.25**	23.77 ± 4.26**
Albumin (g/l)	41.4 ± 4.89	42.0 ± 4.7**	43.1 ± 4.99***	43.3 ± 6.0***	43.1 ± 5.05***
Na+ (meq.l-1)	1.71 ± 0.44	6.55 ± 1.69***	8.35 ± 1.43***	9.35 ± 1.82***	10.11 ± 1.11***
K+ (meq.l-1)	1.74 ± 0.75	2.87 ± 2.72***	3.78 ± 3.55***	4.61 ± 3.68***	5.23 ± 2.5***
Aldosterone (pg/ml)	291.3 ±36.21	293.1 ± 28.45*	297.2 ± 29.24**	302.1 ± 24***	301.4 ± 76.66***
POSM (mosmol/ kg)	251 ±11	258 ± 11***	259 ± 21***	264 ± 32***	265 ± 22***

All Values are expressed as mean±SEM where n=6.
*** p< 0.001, ** p< 0.01, *p< 0.05, Significant difference as compared to the control

Effects of *Barleria Strigosa* leaves on Index Kidney Function

The analysis of the collected urine of rats 24 hours after the administration of a single dose of the *Barleria Strigosa* leaves extract revealed no trace of glucose or albumin. The methanolic extract of *Barleria Strigosa* leaves caused no significant change in the rate of urinary creatinine. The concentration of urea in the urine, on the other hand, was significantly (P 0.01) 250 and 375 mg/kg, respectively (Table 5). Osmotic clearance also significantly increased at the highest dose. The GFR decreased from 1.49 ± 0.41 ml/min in controls to 1.29 ± 0.13 ml/min. The creatinine clearance also decreased.

Table 5: Effect of methanolic extract of *Barleria Strigosa* leaves seeds on Index Kidney Function

Parameters	Groups				
	Control	250 mg/kg	375 mg/kg	500 mg/kg	Furosemide (13mg/kg)
Creatinine (mg/24 h)	23.55 ± 4.83	16.55 ± 1.42***	19 ± 2.54**	23.00 ± 3.50*	18.01 ± 4.25***
CreatC (ml/min)	0.025 ± 0.02	0.022 ± 0.05*	0.020 ± 0.03***	0.018 ± 0.03***	0.019 ± 0.02***
Urea (g/24h)	24.32 ± 3.11	21.31 ± 1.42***	21.01 ± 4.34***	23.64 ± 4.62**	21.54 ± 1.42***
Uosm (mosmol/kg)	187 ± 16	107 ± 17***	110 ± 12***	120 ± 16***	158 ± 18***
GFR (ml/min)	1.49 ± 0.41	1.51 ± 0.31*	1.39 ± 0.11***	1.29 ± 0.13***	1.33 ± 0.16***
Cosm (ml/min)	0.044 ± 0.004	0.042 ± 0.010*	0.043 ± 0.014	0.045 ± 0.007	0.073 ± 0.011***
C H_2O (ml/min)	0.0053 ± 0.011	0.051 ± 0.013	0.054 ± 0.021	0.063 ± 0.006***	0.065 ± 0.012***

All Values are expressed as mean±SEM where n=6.
*** p< 0.001, ** p< 0.01, *p< 0.05, Significant difference as compared to the control
$C_{reat}C$: Creatinine Clearance U_{osm}: Urinary Osmolarity
GFR: Glomerular Filtrations Rate C_{H2O}: Free water clearance

The utilization of herbal medicines and phytonutrients / nutraceuticals continues to rise as a result of increased acceptance and public interest in both developed and developing nations. Herbs and natural plant products are particularly gaining popularity for the management of cardiovascular diseases and associated disorders. The soaring interest in traditional medicine is attributed to the failure of modern medicine to alleviate many chronic illnesses. *The Barleria Strigosa* leaves are highly venerated for their nutritional values and medicinal properties. The leaves of the plant are used as an astringent and diuretic in the treatment of urinary disorders [10, 11].

This study examined the diuretic potential of *Barleria Strigosa* leaves using a methenolic extract of *Barleria Strigosa* leaves. Results of the study showed an increase and acceleration in the elimination of fluid with urinary hypoosmolarity and a moderate increase in natriuretic activity.

These results demonstrate that the methanolic extract of the leaves of *Barleria Strigosa* has a moderate diuretic activity. The increase in natriuresis in response to acute treatment by *Barleria Strigosa* leaves extract may partly explain the increase in diuresis [12, 13]. It showed that the extract's action was time and dose dependent. This can be explained by kinetic differences in the active principle's presence in the extracts and the increment in the urine output in rats might result from the high potassium content in the plant extract. The pH values were also alkaline as compared with control[8]. *Barleria Strigosa* leaves contain a flavonoid as a secondary metabolite, and flavonoids act as diuretics by playing an important role in the RAS system.

Aldosterone hormone measured by radioimmunoassay was slightly increased in animals treated with extract, and the lack of correlation between plasma aldosterone and sodium concentration in the blood as well as in urine seems to imply that aldosterone was not involved in the natriuresis which observed and suggested that stimulation of diuresis by the *Barleria Strigosa* leaves extract could be similar to that of Furosemide [14]. The extract as well as Furosemide caused a urinary increase of Na^+ and Cl^- in rats. The increase of the Na+ excretion tends to reduce GFR by increasing the Na+ load available for Na+/K+ exchange, stimulating further such exchange by hyperaldosteronism (Table 4), which causes a reduction in blood volume [15, 16]. This effect may be due to

the synergistic mechanism of the [HCO_3^-/Cl^-], [HCO_3^+/H^+] and the [Na^+/H^+] antiporter, leading to dieresis. This is a characteristic of high ceiling diuretic. Furosemide acts by inhibiting electrolyte re-absorption in the thick ascending loop of Henle [17].

Collectively, these observations suggest that the *Barleria Strigosa* leaves is not acting as potassium-sparing diuretics. The *Barleria Strigosa* leaves is also unlikely to be acting as thiazide diuretics: these only increase the urinary K^+ level and alter the urinary Na^+/K^+ ratio. But in this study, both urinary Na^+ and K^+ levels were increased without any alteration in the Na^+/K^+ ratio. On the other hand, the diuresis induced by the QL was strong with intensity similar to that of furosemide and accompanied by marked increases in both urinary Na^+ and K^+ levels.

Glomerular filtration measured by creatinine clearance does not vary according to treatment compared to controls, which suggests that the increase in diuresis would rather have a tubular origin as it seems to show the clearance of free water. It is significantly higher in rats that received the plant extract compared to controls ($P < 0.01$) [18]. It acts by inhibiting the reabsorption of Na^+ and Cl^- in the ascending branch of the Henle loop. It also has a peripheral and independent renal vascular action. At this level, it inhibits the reabsorption of sodium. It primarily causes urinary sodium excretion and the elimination of significant chloride. It also ensures the tubuloglomerular feedback inhibition without necessarily increasing the filtration [19].

In the light of the above mentioned study, we can report that the methanol extract of *Barleria Strigosa* leaves. *Barleria Strigosa* leaves is an effective diuretic and also resulted in increased sodium, potassium, and chloride ions in urine; which correlates well with the traditional use of the plant as a diuretic. Nevertheless, it can be suggested that the range of polar phenolic compounds such as flavonoids and tannins in combination with alkaloids might be responsible for the apparent diuretic activity of the plant. The observations showed *Barleria Strigosa* leaves had a diuretic spectrum similar to that of furosemide.

Conclusion

From the above results, it is concluded that *Barleria Strigosa* used by tribals traditionally showed significant diuretic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as diuretic.

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