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Der Pharmacia Lettre, 2011: 3 (4)207-214
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Effect of aqueous extract of *Pergularia daemia* on urine production

Vyas Bhavin A[1*], Vyas Ruchi B[2], Joshi Shrikant V[1], Shah Payal D[1], Santani Dev D[3]

[1]Department of Pharmacology, Maliba Pharmacy College, Gopal Vidyanagar, Tarsadi (Guj), India
[2]Dr. Dayaram Patel Pharmacy College, Bardoli, India
[3]Rofel, Shri G. M. Bilakhia College of Pharmacy, Vapi, India

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ABSTRACT

In present study crude extracts obtain from whole plant Pergularia daemia by using different solvent such as petroleum ether, ethyl acetate, n-butanol and water were subjected to preliminary phytochemical screening for the presence of bioactive constituents. Later promising aqueous extract (200 mg/kg and 400 mg/kg, p.o.) was screened for diuretic property. Diuretic activity of plant was assessed in rats with furosemide as a standard drug using Lipschitzs test. Administration of the aqueous extract leads to diuresis with increase in Na^+ and K^+ level without altering Na^+/ K^+ and Cl^-/ Na^+ + K^+ ratio and slight decrease in pH. Despite of change in urinary excretion of electrolytes the plasma Na^+, K^+ level and hematocrit were not affected by both fractions. The aqueous extract did not appear to have renal toxicity or any adverse effect during the study period. Results also suggest that diuresis produces by the aqueous extract is saluretic rather than aquaretic. Phytochemical analysis of aqueous extract showed presence of flavonoids and phenolic compounds. Thus, the present study revealed that the aqueous extract of P. daemia exhibited significant diuretic activity in the tested model and support the pharmacological credence to the folkloric and ethnomedical uses of P. daemia.

Key Words: *Daemia extensa*, Diuretic activity, Glomerular filtration rate, conductivity.

INTRODUCTION

Plant medicine is used worldwide in the traditional treatment of some renal diseases and for dieresis [1]. The plant *Pergularia daemia* (Family: Asclepiadaceae) is known as “Uttaravaruni” in Sanskrit and “Utranajutuka” in Hindi. In ethanomedicinal practices the traditional healer use *Pergularia daemia* (Asclepiadaceae) as anthelmentic, emetic, thermogenic, expectorant, antipyretic and laxative. Leaves juice is given in catarrahal affections, asthma, and infantile

diarrhoea and is applied to inflammatory swelling in combination lime [2] . Aerial parts of the plant used for snake bite [3]. Latex of this plant used for boils and sores [4] . Fresh roots of plant used as an abortifacient [5] and used to treat gonorrhea [6]. The latex or a decoction of the roots is used in many countries as a medicine to treat several illnesses, such as veneral diseases, arthritis, muscular pains, asthma, rheumatism, snake-bites. The latex may also be used as a fish poison [7] and toothache [8]. Plant has been documented for antidiabetic, [9] wound healing [10], hepatoprotective activity [11], antibacterial[12] , anti-urolithiatic, diuretic [1] and antifertility [13]. Plant has been documented for presence of presence of triterpenes, saponins cardenolides and alkaloids [14], while Anajanyulu et al. (1998) [15] reported the presence of triterpenes and steroidal compound. In attendance, study was undertaken to examine and to validate the diuretic properties of aqueous extract of *Pergularia daemia* and to verify or contradict the claims made in the traditional medicine.

MATERIALS AND METHODS

The plant material was bought from Botanical Source of India, Jodhpur. The dried powdered plant material was extracted with different solvent such as petroleum ether, ethyl acetate, n-butanol and water in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure to obtain semi solid mass. The aqueous extract was used in present for diuretic activity. The dose of extract selected on the basis of our previous work [1]. Furthur, the extract was screened for various constituents (alkaloids, saponins, tannins, anthraquinones, sterol, flavonoids, terpenoids, glycosides, simple sugars) using standard protocol. [16]

Animals

Swiss albino mice of either sex weighing between (20 - 25 g) or Albino Wistar rats of the either sex (200 - 250 g) were used for the present study. Animals were housed in groups of five under standard laboratory conditions of temperature (25 ± 20 C) and 12/12 hr light/dark cycle. They were provided with standard pellets and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Diuretic Activity

The method described Wiebelhaus et al. 1965 [17] was employed, with modification, for the assessment of diuretic activity. Healthy albino rats of either sex (160-200 g) were divided into twelve groups of five animals each. They were fasted 18 hours prior to the test, with free access to water. On the day of the experiment, animals were given 25ml/kg of body weight normal saline orally. Group A received vehicle (0.2 ml of 5% tween 80) and served as control group. Group B: Furosemide 100 mg/kg; Group C: Water – 200 mg/kg; Group D: Water – 400 mg/kg. All drugs/vehicle were administered orally (p.o.). Immediately after dosing, the rats were placed in the metabolic cages with special provision to collect faeces and urine. Animals were kept at room temperature of 35 ± 1^0 C throughout the experiment. Urine excreted for the next 5 h was collected and the total 5 h urine volume for each rat was compared with the volume of urine produced after the administration of normal saline.

The volume of urine excreted during 5 h for each animal in the group is expressed as the percent of the liquid (normal saline) administered. This percentage gives a measure of urinary excretion

independent of the animal weight. The ratio of urinary excretion in the test group to urinary excretion in the control group is used as a measure of the diuretic action for the given dose of the drug. As the diuretic action is prone to variability, a parameter known as diuretic activity was calculated instead. To obtain the diuretic activity, the diuretic action of the extract is compared to that of the standard drug in the test group [18].

$$\text{Percentage of saline load excreted} = \frac{\text{volume of urine}}{\text{volume of saline load}} \text{ X } 100$$

$$\text{Urinary excretion} = \frac{\text{total urinary output}}{\text{total liquid administered}} \text{ X } 100$$

$$\text{Diuretic action} = \frac{\text{urinary excretion of treated group}}{\text{urinary excretion of control group}}$$

$$\text{Diuretic activity} = \frac{\text{diuretic action of test drug}}{\text{diuretic action of standard drug}}$$

The parameters taken to study were pH, Urine and plasma electrolytes, Plasma urea level, Hematocrit, GFR, Urine conductivity

Diuretic action:
Diuretic action of plant was estimated by ratio of urinary excretion of treated group by urinary excretion of standard drug

Urine and plasma electrolytes
Blood was collected in capillary tubes containing ethylenediamine tetraacetic acid by retro-orbital puncture under light diethyl ether anesthesia. Plasma was obtained by centrifugation (600×g at 4 ◦C), and stored at −20 ◦C until analyzed. Plasma and urinary levels of sodium and potassium were quantitated by flame spectrophotometry, while Cl- concentration will be determined titrimetrically.

Plasma urea level
Blood was collected in capillary tubes containing ethylenediamine tetraacetic acid by retro-orbital puncture under light diethyl ether anesthesia. The plasma urea level was estimated by DAM method.

Hematocrit
Blood sample at the end of experiment were collected and was analyze by autoanalyzer for the estimation of hematocrit.

Urine conductivity
Conductivity of urine was measured by conductometer.

GFR

Glomerular filtration rate was evaluated by the clearance of creatinine. Concentration of creatinine in plasma and urine was determined by the Jaffe alkaline picrate method. For urinary creatinine excretion 24 h urine was collected and its volume measured. Glomerular filtration rate (GFR) was estimated from creatinine clearance (CCr =UCr ×V/PCr, where UCr: urinary excretion of creatinine and PCr: plasma level of creatinine, V: volume of urine).

Statistical Analysis

The data were expressed as mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnett's multiple comparisons test for parametric data. Data were considered statistically significant if *P* value <0.05.

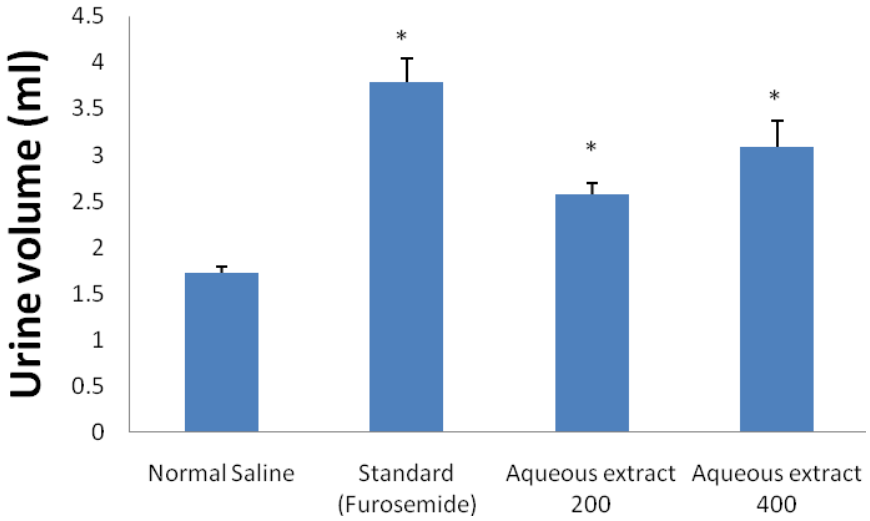
RESULTS

Phytochemical analysis

Result of preliminary phytochemical analysis conducted on aqueous extract of *Pergularia daemia* indicates presence of flavonoids and phenolic compounds (Table 1).

Table 1: Total phenolic and flavonoid content of aqueous extract *Pergularia daemia*

	Total phenolic content (mg of gallic acid equivalents / g of dry extract)	**Total flavonoid Content** (mg of catechin equivalents / g of dry extract)
Aqueous extract of *P. daemia*	15.62 ± 0.24	2.03 ± 0.08



Each value was represented as mean ±SEM. n = 6, P < 0.001 when compared to the control group (one-way ANOVA followed by Dunnett's test)*

Figure 1 : Effects of Aqueous extract of *Pergularia daemia* on diuresis in the rat

Urine volume, electrolyte levels

The results showed that aqueous extract (200 and 400 mg/kg), increases urine output (Figure 1), which is expressed as the percentage of saline load excreted, and increased the renal excretion of Na^+ and K^+, without altering the Na^+/K^+ ratio and ion quotient ($Cl^-/Na^+ + K^+$) as compared to control group with acidification of urine (Table 2). The diuretic action of furosemide and water fraction (400 mg/kg) was found to be 2.19 and 1.85 respectively as compared with 1 of control group (Table 3). Diuretic activity of alcoholic extract was found to be 0.86 of aqueous extract (Table 4).

Table 2 : Effects of ethanolic extract and various fractions of *Pergularia daemia* on electrolyte excretion and pH in the rat

Treatment	Dose (mg/kg)	Urine volume (ml)	Electrolyte Concentration (meq/l)					pH
			Na^+	K^+	Cl^-	Na^+/K^+ ratio	$Cl^-/Na^+ + K^+$	
Normal Saline	25 ml/kg	1.73±0.06	84.52±0.48	55.71±1.23	92.70±1.06	1.51	0.66	7.1±0.2
Furosemide	100	3.79±0.26**	139.11±0.61*	89.42±0.93*	187.39±0.57*	1.55	0.82	6.3±0.12
Water Fraction	200	2.58±0.11**	122.82±1.21*	79.61±0.94*	163.84±1.36*	1.54	0.81	6.4±0.16
	400	3.08±0.29**	129.89±0.47*	83.48±0.53*	179.23±0.49*	1.55	0.84	6.6±0.09

n = 6, Each value was represented as mean ±SEM. n = 6, P < 0.05, ** P < 0.001 when compared to the control group (one-way ANOVA followed by Dunnett's test)*

Table 3 : Effects of Aqueous extract of *Pergularia daemia* the percent excretion of administered saline load in the rat

Treatment	Dose (mg/kg)	Percentage of saline load excreted	Diuretic action
Normal Saline	25 ml/kg	38.44 ± 4.36	1
Furosemide	100	84.22 ± 6.78**	2.19
Aqueous extract	200	57.33 ± 3.49*	1.49
	400	71.34 ± 4.87**	1.86

*Each value was represented as mean ±SEM. n =6, * P < 0.05, ** P < 0.001 when compared to the control group (one-way ANOVA followed by Dunnett's test)*

Table 4 : Diuretic activity of Aqueous extract of *Pergularia daemia*

Treatment	Dose (mg/kg)	Diuretic activity
Aqueous extract	200	0.68
	400	0.86

Each value was represented as mean ±SEM. n =6

Effects on plasma urea levels, plasma electrolyte, hematocrit and conductivity

No remarkable changes were noticed on plasma urea levels, plasma electrolytes and hematocrite after aqueous extract of *P. daemia* (200 and 400 mg/kg) and furosemide treatment (Table 5). On the contrary, Furosemide and test drug (400 mg/kg) showed significant increase in urine conductivity (Table 5).

Table 5 : Effect of aqueous extract of *P. daemia* and Furosemide on plasma urea levels (mmol/l), plasma electrolytes, Hematocrit and urine conductivity

Treatment	Dose (mg/kg)	Urea (mmol/L)	Plasma electrolyte level		Hematocrit%	Urine Conductivity
			Na+ (mmol/l)	K+ (mmol/L)		
Normal Saline	25 ml/kg	7.26 ± 0.64	145.61 ± 1.36	5.56 ± 0.23	35.61 ± 4.86	12.62 ± 1.43
Furosemide	100	7.39 ± 0.19	147.58 ± 1.81	5.29 ± 0.36	41.24 ± 2.54	15.01 ± 0.21*
Aqueous extract	200	7.48 ± 0.38	142.85 ± 1.28	5.20 ± 0.31	34.63 ± 4.19	13.73 ± 0.18
	400	7.50 ± 0.12	145.37 ± 1.69	5.41 ± 0.19	37.34 ± 3.32	14.29 ± 0.91#

*Each value was represented as mean ±SEM. n =6, * P < 0.05, # P < 0.001 when compared to the control group (one-way ANOVA followed by Dunnett's test)*

Effect on plasma levels and urinary excretion of creatinine and glomerular filtration rate

Neither of the test drug nor furosemide had any significant effect on plasma creatinine levels as well as Urinary creatinine levels (Table 6).

Table 6 : Effect of Aqueous extract of *P. daemia* and Furosemide on Urinary Creatinine, Serum Creatinine and GFR

Treatment	**Dose (mg/kg)**	**Urinary Creatinine level (mg/dl)**	**Serum Creatinine level (mg/dl)**	**Creatinine Clearance (ml/min) GFR**
Normal Saline	25 ml/kg	88.69 ± 6.12	0.60 ± 0.041	1.81 ± 0.23
Furosemide	100	92.36 ± 8.46	0.68 ± 0.084	1.88 ± 0.19
Water	200	96.86 ± 6.71	0.64 ± 0.067	1.84 ± 0.38
Fraction	400	85.94 ± 7.08	0.59 ± 0.38	2.03 ± 0.40

Each value was represented as mean ±SEM. n =6

DISCUSSION

Earlier studies of diuretic agents have ascertained it is beneficial to pre-treat or "prime" the test animal with assorted fluids. The administration of saline has been found to be requisite to produce a graded response in the male rat with escalating dosage of aminophylline [19]. As diuretics are utilized clinically in the management/treatment of edema, they would appear to be most imperative to prove efficiency in the presence of electrolyte and water. In the present investigation, we examined the diuretic potential of *P. daemia* in rats primed with saline. The results showed that the aqueous extract increased urine output, which is expressed as the percentage of saline load excreted, as compared to control group. Further, we studied aqueous extract of *P. daemia* for evaluation of probable mechanism as diuretic agent. Diuretics have two separate connotations: increased urinary out put *per se* and net loss of solute and water. These two processes are involved in the inhibition of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and as a consequence, promote the formation of urine [20]. The results of present study show that test extract at a dose of 200 and 400 mg/kg administered orally produced an increase in urinary excretion and urinary sodium loss but no effect on urinary potassium as compared to control and standard drug treated groups.

Some herbal diuretics induce diuresis by stimulating the thirst center in the hypothalamus and thereby enhancing the fluid intake [21]. Such a mode of action is unlikely to be operative with the test extract since the rats had no access to fluid intake during the 5 h experimental period. In addition, earlier studies revealed that diuretic drug causes increase in GFR due to either a direct effect on arterial pressure or glomerular blood flow [22] or by decreasing renal perfusion pressure [23,24] produces diuretic action, indicated by increase in GFR. Such a mode of action is unlikely to be operative with test drug since administration of the aqueous extract caused the diuretic response, without affecting GFR. The aqueous extract-induced diuresis was significant with raised urinary Na + levels and was not accompanied with a reduction in urinary K+ levels. In addition, there was no alkalization of urine and without alteration in Na+/K+ ratio i.e. aldosterone secretion index. These data indicate that the drugs/fractions are not acting as potassium sparing diuretics [25-27]. The present test extract was also unlikely to be acting as thiazide diuretics: they only increase urinary K+ level and alter the urinary Na+/K+ ratio [26]. But in this study, both urinary Na+ and K+ level were increased without any alteration in Na+/K+ ratio. The ratio Cl-/ Na+ + K+ (ion quotient) is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios, slight to strong carbonic anhydrase inhibition can be assumed [28]. The present study indicates the test extract at dose 200 and 400 mg/kg had no significant effect on Cl-/ Na+ + K+ (index for carbonic anhydrase inhibitory activity), excluding carbonic anhydrase inhibitory activity of both the fractions. In this study, marked natriuresis (in terms of increased urinary Na + level and sodium saluretic index) was evident possibly because of the inhibition of Na + reabsorption in the nephron [29], thereby increasing the urinary output. The diuresis induced by aqueous extract of *P. daemia*, was similar to that of furosemide and was accompanied by marked increases in both urinary Na+ and K+ levels. Further, the urine was slightly acidified. These characteristics strongly suggest the extract is acting as loop diuretic. Loop diuretics inhibit the Na+/K+/Cl- co-transporter system in the thick ascending loop of nephron, thereby increasing natriuresis and kaleuresis [25, 26] and also cause acidification of urine [26].

Since the conductivity value is an indirect measure of the electrolyte concentration in the urine, in the present study extract at 400 mg/kg showed increase in conductivity suggesting the diuretic effect of the extract to be saluretic rather than aquaretic, the latter being the typical effect of diuretics of plant origin. The test extract did not affect plasma urea levels and hematocrit which indicates that the rapid physiological regulation of these important parameters was not altered after extract treatment. Collectively, these observations indicate that the aqueous extract of *P. daemia* possesses oral diuretic activity, which is mediated via inhibition of the Na+/K+/Cl- co-transporter system i.e. loop diuretic-like action.

Acknowledgement
Authors are thankful to Dr. D. R. Shah, Principal, Maliba Pharmacy College, Tarsadi, India for providing necessary facilities to carry out the research work.

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