ORIGINAL ARTICLE

Diuretic activity of aqueous extract of *Spilanthes paniculata* flower in rats

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Objectives: To evaluate the diuretic activity of aqueous extract of *Spilanthes paniculata* (SpE) flowers in rats. **Materials and Methods:** The three different doses of aqueous extract of SpE flowers (aqueous SpE) (100 mg/kg, 300 mg/kg, 500 mg/kg) and standard furosemide (10 mg/kg) were orally administered to rats. The various analytical parameters like urine volume, pH, density, conductivity, Na^+ and K^+ content were measured in the urine of saline loaded rats. **Results:** Treatment with three different doses of aqueous extract of SpE flowers (100 mg/kg, 300 mg/kg, 500 mg/kg p.o.) and standard furosemide (10 mg/kg) has significantly increased the urine volume, conductivity, Na^+ and K^+ content as compared to control group. The pH of urine was found to be increased by aqueous extract at 500 mg/kg, whereas decreased by aqueous extract at 100 mg/kg, 300 mg/kg, and standard furosemide. The density of urine shows significant similarity in the treated group compared to control group. **Conclusion:** The results suggest that the aqueous extract of SpE flowers presents a notable diuretic effect at different doses of (100 mg/kg, 300 mg/kg, and 500 mg/kg, p.o.) which is associated with marked increase in both urinary Na^+ and K^+ levels. The aqueous extract (500 mg/kg) has shown the most pronounced diuretic effect and may act as loop diuretic.

Key words: *Asteraceae*, diuretic activity, *Spilanthes paniculata*

INTRODUCTION

India is a repository of herbal medicines, and there are evidences of herbs being used in the treatment of diseases and for revealing various body systems in all ancient civilizations.[1] 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 its basic health-care needs.[2]

Spilanthes paniculata (SpE) Wall. ex DC, belongs to family *Asteraceae* is an important medicinal plant with rich source of therapeutic constituents and is commonly known as toothache plant [Figure 1]. The genus *Spilanthes* (Asteraceae) comprises 30 species and additional intraspecific taxa that are mainly distributed in the tropical and subtropical regions around world.[3] It exhibits analgesic, strong larvicidal activity on *Anopheles stephensi* Liston, *Anopheles culicifacies*, antimicrobial and cytotoxic activity,[4] toothache.[5]

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In particular, this species is famous as a folklore remedy for throat infections, and paralysis of tongue.[6] The ethnobotanical uses of *Spilanthes* species are also said popular remedy for stammering in children in Western India, stimulant, sialogoue, anesthetic, powerful insecticide, antioxidant, and hepatoprotective.[3,5-7]

Various pharmacological experimental studies have been carried out with *S. acmmella* another species of same genus. The chloroform, ethyl acetate, and methanol extracts prepared from the aerial part of *S. acmmella* shows vasorelaxant and antioxidant activities.[8] A previous study has reported the presence of stigmasterol, sitosterol-o-β-D-glucoside in *S. paniculata* plant.[9]

The two widely used diuretics thiazides and furosemide have been associated with numerous adverse effects.[10] Hence, there is a requirement for novel diuretics such as plant-based substances which are considered to be relatively safe, possessing lower potential for adverse effects. Many indigenous drugs have been claimed to have diuretic effect in the Ayurvedic system of medicine, but they were not properly investigated.[11]

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Figure 1: *Spilanthes paniculata* Wall. ex DC

Although, SpE is well-recognized in Indian traditional medicine for possessing diuretic effect, no scientific data have been published supporting the claimed ethnomedical action. Hence, the main aim of present study was to evaluate the diuretic activity of aqueous extract of SpE flower in rats and its possible mechanism.

MATERIALS AND METHODS

Plant Material

The SpE flowers were procured from the local area of Mazalgaon in a location of Beed (Maharashtra). The plant material was identified, confirmed, voucher herbarium specimens were submitted and authenticated (no. 0730) by in the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, (M. S.).

Preparation of the Extract

The fresh flowers of SpE plant were weighed (300 g) and homogenized in distilled water using a domestic mixture for 10 min and filtered through eight layers of muslin cloth. The resulting brown-colored filtrate was kept for removal of water under reduced pressure; the remaining concentrated extract was stored airtight container in deep freezer. The yield obtained from the aqueous extraction process was found to be 9.36% w/w.

Drugs

Furosemide (Aventis Pharma Ltd.,) was used as the reference diuretic drug. Other reagents used in the study were of analytical grade.

Phytochemical Screening

Chemical tests were carried out on aqueous extract using standard procedures to identify the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins, and steroids.[12]

Experimental Animals

Albino Wistar rats of either sex weighing 100–180 g were used for the study. The animals were maintained in sanitized polypropylene cages containing sterile paddy husk as bedding under controlled conditions of temperature (23°C ± 2°C), humidity (50% ± 5%), and 12 h light-dark cycles. All the animals were acclimatized for 7 days before the study. They had free access to standard pellets as basal diet and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of our college. The approval no. was (Committee for the Purpose of Control and Supervision of Experiments on Animals) [CPCSEA]/IAEC/P'col/07/2010-11/23 and the experiment was conducted according to prescribed guidelines of (CPCSEA), Government of India.

Evaluation of Acute Toxicity

In this experiment, two groups of Wistar rats (n = 3) were used. Animals were fasted overnight with water *ad libitum*, and food was withheld for 3–4 h after oral administration of the extracts. One group of animals was treated with starting dose of 2000 mg/kg body weight. Orally and the maximum dose of 5000 mg/kg body weight was administered to the second group. Another group was treated with normal saline. Animals were observed individually after dosing, during the first 24 h, with special attention given during the first 4 h (OECD guidelines, 2006). Clinical signs which include changes in skin fur, eyes and mucous membranes, behaviors, effect on passivity, body weight, and water intake were observed. As per OECD guidelines, the substance might be considered to have an LD50 value above 2000 mg/kg and 5000 mg/kg body weight. It was found that the aqueous extract of SpE was safe at limit dose 5000 mg/kg and 2000 mg/kg; no mortality was seen with these doses. Therefore, the dose selected was 1/10th of this dose, that is, 500 mg/kg was used in the present study for the extract of SpE flowers.

Experimental Design

Thirty rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally administered with 5 ml/100 g body weight of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water load. 45 min later, these rats were randomly assigned into five groups (N = 6 per group) and treated orally.

- Group I: Animals served as normal control and received vehicle (distilled water, 10 ml/kg, p.o.)
- Group II: Animals were pretreated with the furosemide, (10 mg/kg, p.o.) a standard drug
- Group III: Animals were pretreated with the aqueous SpE (100 mg/kg, p.o.)
- Group IV: Animals were pretreated with the aqueous SpE (300 mg/kg, p.o.)

- Group V: Animals were pretreated with the aqueous SpE (500 mg/kg, p.o.).

Collection of Urine Samples

Each of these rats was individually placed in metabolic cages, and cumulative urine output was determined at 2 h intervals for 8 h. The urine samples were collected in measuring cylinders.

Analytical Procedure

The urine collected from all group were subjected to the following investigations, cumulative urine excretion volume in relation to body weight and expressed as ml/100 g body weight, pH by electronic pH meter, density was determined by pycnometer, conductivity was determined by electronic conductometer, Na^+ and K^+ levels by flame photometry, Na^+/K^+ ratio was then computed, and diuretic index was determined. The color of urine was also noted.[13-16]

Statistical Analyses

The mean ± standard error of the mean (SEM) values was calculated for each group. A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests was used for statistical analysis. $P < 0.05$ were considered statistically significant. The entire statistical analysis was performed using a statistical package, GraphPad Instat version 3 (Graph Pad Software, Inc., California, USA).

RESULTS

Phytochemical Screening

Phytochemical screening revealed the presence of medicinally active constituents. The aqueous extract of SpE flowers was found positive for the presence of flavonoids and steroids. However, the extract was devoid of alkaloid, glycosides, tannins, and phenolic compounds.

Acute Toxicity

The oral administration (100 mg/kg, 300 mg/kg, 500 mg/kg) of aqueous extract of SpE flower in rat did not produce acute toxicity, as evidenced by absence of mortality in the animals during the study period.

Diuretic Activity

Different parameters were analyzed for possible diuretic action of three doses of aqueous extract of SpE flower and furosemide (standard drug) on excreted urine volume, pH, density, conductivity, Na^+ and K^+ ions content, diuretic index. Treatment with standard furosemide (10 mg/kg) and the different doses of aqueous SpE (100 mg/kg, 300 mg/kg, 500 mg/kg) has significantly increased the urine volume (ml/100 g/8 h), excreted urine conductivity (mS/cm), urine electrolyte Na^+ ion (ppm) and K^+ ions (ppm) as compared to control group. Aqueous SpE 500 mg/kg has significantly increased the pH of urine, whereas aqueous SpE 100 mg/kg, 300 mg/kg, and standard furosemide have significantly decreased the pH of urine. The density (g/ml) of excreted urine shows significant similarity in treated groups compared to normal control group. No significant change was observed in the density of excreted urine in all treatment groups as shown in Tables 1 and 2.

Effect of Aqueous Extract of *Spilanthes paniculata* Flowers on Excreted Urine Volume

Different doses of aqueous extract (100 mg/kg, 300 mg/kg, 500 mg/kg) of SpE flowers and standard

Table 1: Effect of aqueous SpE flowers and furosemide on excreted urine (volume, pH, density, and diuretic index) of treated rats

Groups	Urine volume (ml/100 g/8 h)	pH	Density (g/ml)	Diuretic index
Normal control	4.02±0.35	7.46±0.05	0.98±0.002	1.00
Furosemide (10 mg/kg)	7.79±0.27†	6.18±0.07†	0.98±0.001	1.93
Aqueous SpE (100 mg/kg)	6.27±0.37†	7.06±0.07†	0.98±0.001	1.55
Aqueous SpE (300 mg/kg)	6.39±0.41†	6.98±0.04†	0.98±0.001	1.58
Aqueous SpE (500 mg/kg)	6.95±0.41†	7.93±0.14†	0.98±0.001	1.72

Each value represents the mean±SEM (n=6), data were analyzed by one-way ANOVA followed by Dunnett's test. Comparisons were made with control group versus all treated groups. †Represents statistical significance at P<0.01. Aqueous SpE – Aqueous extract of *Spilanthes paniculata*, SEM – Standard error of mean

Table 2: Effects of aqueous SpE flowers and furosemide on excreted urine (conductivity and Na^+, K^+ ions) of treated rats

Group	Conductivity (mS/cm)	Na^+ ion (ppm)	K^+ ions (ppm)	Na^+/K^+
Normal control	13.80±0.3†	327.15±5.17	284.91±7.90	1.14
Furosemide (10 mg/kg)	18.01±0.46†	883.50±7.51†	592.93±7.79†	1.49
Aqueous SpE (100 mg/kg)	15.84±0.31†	577.36±5.05†	390.81±8.72†	1.47
Aqueous SpE (300 mg/kg)	16.85±0.19†	766.51±5.92†	502.96±6.52†	1.52
Aqueous SpE (500 mg/kg)	17.46±0.38†	875.11±6.22†	548.25±7.10†	1.59

Each value represents the mean±SEM (n=6), data were analyzed by one-way ANOVA followed by Dunnett's test. Comparisons were made with control group versus all treated groups, †Represents statistical significance at P<0.01. Aqueous SpE – Aqueous extract of *Spilanthes paniculata*; SEM – Standard error of mean

furosemide (10 mg/kg) treatment has significantly increased the urine volume as compared to normal control group ($P < 0.01$).

Effect of Aqueous Extract of *Spilanthes paniculata* Flowers on Excreted Urine pH

Different doses of aqueous extract (100 mg/kg, 300 mg/kg) of SpE flowers and standard furosemide (10 mg/kg) treatment have significantly decreased the urine pH as compared to normal control group ($P < 0.01$). However, the aqueous extract (500 mg/kg) of SpE flowers has significantly increased the urine pH as compared to normal control group ($P < 0.01$).

Effect of Aqueous Extract of *Spilanthes paniculata* Flowers on Excreted Urine Density

Different doses of aqueous extract (100 mg/kg, 300 mg/kg, 500 mg/kg) of SpE flowers and standard furosemide (10 mg/kg) show similarity as compared to normal control group and have not produced significant change in density of urine as compared to normal control group.

Effect of Aqueous Extract of *Spilanthes paniculata* Flowers on Excreted Urine Conductivity

Different doses of aqueous extract (100 mg/kg, 300 mg/kg, 500 mg/kg) of SpE flowers and standard furosemide (10 mg/kg) treatment have significantly increased the urine conductivity as compared to normal control group ($P < 0.01$).

Effect of Aqueous Extract of *Spilanthes paniculata* Flowers on Excreted Urine Na^+ Ions

Different doses of aqueous extract (100 mg/kg, 300 mg/kg, 500 mg/kg) of SpE flowers and standard furosemide (10 mg/kg) treatment have significantly increased the urine Na^+ ions as compared to normal control group ($P < 0.01$).

Effect of Aqueous Extracts of *Spilanthes paniculata* Flowers on Excreted Urine K^+ Ions

Different doses of aqueous extract (100 mg/kg, 300 mg/kg, 500 mg/kg) of SpE flowers and standard furosemide (10 mg/kg) treatment have significantly increased the urine K^+ ions as compared to normal control group ($P < 0.01$).

Each value represents the mean ± SEM ($n = 6$), data were analyzed by one-way ANOVA followed by Dunnett's test. Comparisons were made with control group versus all treated groups, represent statistical significance at $P < 0.01$.

DISCUSSION AND CONCLUSION

This study investigated the diuretic potential of aqueous extract of SpE flowers. The results showed that the highest dose of aqueous extract of flowers tested possesses strong diuretic activity when given orally in a single dose. The present findings suggest for the first time that ethnopharmacological effect of three different doses of aqueous SpE (100 mg/kg, 300 mg/kg, 500 mg/kg) is probably mediated through its ability to cause a significant increase in urine volume, sodium and potassium excretion, without interfering with other parameters related to renal functions, for example, creatinine.

The aqueous SpE has induced strong diuresis and was not accompanied with a reduction in urinary K^+ levels. Further, there was no alkalization of urine. Collectively, these observations suggest that the aqueous SpE is not acting as potassium-sparing diuretics. The aqueous SpE is also unlikely to act as thiazide diuretics, these only increase the urinary K^+ level and alter the urinary Na^+/K^+ ratio.[17,18] 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 aqueous SpE showed a notable dose-dependent increase in the urinary volume and urinary electrolyte (Na^+ and K^+) ions excretion. The diuresis induced by the aqueous SpE was strong, and the intensity was similar to that of furosemide and accompanied by marked increases in both urinary Na^+ and K^+ levels. These features strongly suggest that the aqueous SpE may act as a loop diuretic. Loop diuretics are the most powerful of all diuretics and these inhibit the $Na^+/K^+/Cl^-$ co-transporter system in the thick ascending loop of the nephron, thereby increasing natriuresis and kaliuresis. These diuretic also cause acidification of urine.[19] Further, the onset of the diuretic activity of the aqueous extract was extremely rapid as observed with clinically used synthetic loop diuretics. Interestingly, in spite of the heavy loss of urinary Na^+ and K^+, there was a significant reduction in the osmolarity of urine in aqueous extract treated rats. Thus, it is possible that the aqueous extract, in addition, may impair the basal secretion of antidiuretic hormone (ADH) and/or diminished the responsiveness of uriniferous tubules to the action of ADH and the inhibition of ADH causes polyurea with low osmolarity.

Phytochemically, SpE flowers contain stearic acid, stigmasterol, sitosterol-o-β-D-glucoside, and amino acids.[5,9] Amino acids are resorbed in the proximal convoluted tubules of nephrons[17,18] and cannot function as diuretics. The active constituents responsible for the diuretic effects of the SpE flowers are, so far not known, therefore, we hypothesized that the presence of polar compounds such as flavonoids and steroidal lactones might be responsible for diuretic activity. The effect may be produced by stimulating regional blood flow or initial vasodilation or by producing inhibition of the tubular reabsorption of water and anions, with the result in both cases being diuresis.[10]

Loop diuretics are clinically used in patients with salt and water overload due to conditions such as pulmonary edema, heart failure ascites, and hypertension.[19] As aqueous SpE has a similar mode of action so it may be useful as a nontoxic natural therapeutic agent in the treatment of such conditions by traditional practitioners. The onset of the diuretic action of the aqueous extracts was extremely rapid, and it also had a fairly long duration of action. However, there is one major limitation, an increased risk of hypokalemia as with other therapeutically used loop diuretics. In addition, these SpE flowers may be useful in the treatment of bacterial urinary infections based on the certain degree of antimicrobial activity.[20] Diuretic activity of *Spilanthes acmella* flowers in rats[21] that shows this aqueous extract of SpE flowers also have diuretic activity in rats. The phytochemical investigation carried out in this study has revealed the presence of flavonoids and steroids was found to be present in the aqueous extract of *S. paniculata* flowers. The same phytochemicals were found to be present in the earlier study, where it was reported that flavonoids and steroids in aqueous extract of *S. paniculata* flowers are present. Tannins, phenolic compounds were found to be absent in aqueous extract.[7]

It was also concluded that the SpE flowers show diuretic effect, which may due to an marked increase in both urinary Na^+ and K^+ levels, from the three different doses of aqueous SpE (100 mg/kg, 300 mg/kg, 500 mg/kg) and aqueous SpE (500 mg/kg) has shown the most pronounced diuretic effect.

In summary, revisiting all the data presently available on the SpE flowers extracts, a very interesting and most pronounced loop diuretic effect can be noted. Hence, SpE flowers were proved to be one of the herbal remedies as a diuretic.

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Conflicts of Interest
There are no conflicts of interest.

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