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Research Article

PRELIMINARY STUDIES ON DIURETIC EFFECT OF *SPILANTHES ACMELLA* LEAVES EXTRACTS IN RATS

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ABSTRACT

Petroleum ether, chloroform and alcohol extracts of leaves of *Spilanthes acmella* were evaluated for its diuretic activity using modified method of Rao. The animals were grouped into different groups of six animals each. All the animals received priming dose of 0.9% sodium chloride solution (20 ml/kg body weight p.o.). The first group of animals, served as control, received normal saline (20 ml/kg body weight p.o.); the second group received the standard drug frusemide (10 mg/kg body weight p.o.) in 0.9% sodium chloride solution and The other three groups received petroleum ether, chloroform, and alcohol extracts of *Spilanthes acmella* leaves in a dose of 500 mg/kg body weight suspended in 0.9% sodium chloride solution (p.o.). The urine volume was recorded for all the groups for 5h. and electrolyte concentration (Na^+, K^+ and Cl^-) were measured. The extracts showed increase in total urine volume and electrolytes excretion (sodium Na^+, potassium K^+ and chloride Cl^-). So, Out of the different extracts, the alcohol extract (500 mg/kg) significantly and markedly increased the urine output ($p < 0.01$). The pattern of diuresis induced by the alcohol extract was almost similar to that produced by the frusemide. These findings suggest the possible traditional use of this plant in hypertension as diuretics are used in the management of hypertension.

Keywords: *Spilanthes acmella,* Frusemide, Diuretic activity, Electrolyte excretion.

INTRODUCTION

Diuretic agents have very wide application in the treatment of various chronically diseases associated with edema. They are generally prescribed for the treatment of hypertension, congestive heart failure, glaucoma, diabetes insipidus and liver aliments. The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties.[1] *Spilanthus acmella* is commonly known as akarkara, is used medicinally in Indochina, Philippine islands, Lareunion and Madagascar; *Spilanthus acmella* introduced from brazil and often cultivated in garden in many part of India. Leaves are opposite, broadly ovate-lanceolate, 2.5-5 by 1.3-3.8cm, sub obtuse, irregularly crenate-serrate or sometime entire, glabrous or nearly so, base usually acute petioles 0.6-1.6cm long, pubescent. Trichomes present on both the surfaces. Upper surface is darkening than the lower one. Midribs prominent on lower surface. Stems are glandular and hairy with pungent taste. The whole plant is acrid in taste.[2] The leaves are used as immunomodulatory, adaptogenic, diuretic, tooth paste, lithotriptic, antiscorbitic, sailagogine, antibacterial, tonic and digestive.[3-6] The leaves contain alkaloids, carbohydrates, pungent amide, tannins, steroids, carotenoids, provitamin A, α-carotene and β-carotene, essential oils, sesquiterpenes, and amino acids etc.[7-13] Preliminanary studies have reported as diuretic[14], antiinflammatory and analgesic[15], vasorelaxant and antioxidant.[16] However, no systematic pharmacological studies have been carried out in order to confirm its diuretic activity. Hence, in the present study diuretic activity of petroleum ether, chloroform and alcohol extracts of leaves of *Spilanthes acmella* was investigated to justify the rationale behind using this plant as diuretic in hypertension. The present investigation was undertaken to confirm traditional medicinal use of the plant.

MATERIALS AND METHODS

Plant Material

Leaves of *Spilanthes acmella* (Family-Compositae) collected from local areas of Hubli, Karnataka (India) and authenticated by Dr. Ganesh Hegde, Professor and Head, Dept. of Botany, Karnataka University, Dharwad, Karnataka and voucher specimen has been deposited at the herbarium for further reference.

Processing of plant material[17]

Dried coarse powder (40-mesh) leaves (500g) of *Spilanthes acmella* was subjected to successive extraction in a soxhlet apparatus using petroleum ether (60-80°C), chloroform and alcohol. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. Filter it rapidly through whatman No. 1 filter paper. The extract was concentrated to ¾ of its original volume by rotary evaporator. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and dried over a desicator. The yield was 11.40% w/w, 5.32% w/w and 17.52% w/w for petroleum ether, chloroform, and alcohol extract, respectively were subjected to preliminary phytochemical analysis.

Phytochemical investigation[18]

Qualitative Phytochemical tests were done by Harbone method for different extracts of *Spilanthes acmella* leaves to identify the various phytoconstituents. The results of preliminary phytochemical investigation are shown in Table 1.

Drugs and chemicals

All the drugs, chemicals, and reagents were procured from S.D. Fine Chemicals, (Mumbai, India). All the chemicals were of analytical grade.

Acute toxicity studies

Healthy albino mice of either sex weighing 25-30g, maintained under controlled conditions of temperature (20 –25^{0}C) and humidity (55%) were used for toxicity study as per Up & Down or Staircase method.[19] The maximum no-lethal and the minimum lethal dose are thus determined using only about 10 mice, once the approximate LD_{50} or the range between the maximum non-lethal and minimum lethal dose is found, a final and more reliable LD_{50} assay is planed using at least 3 or 4 dose levels within this range with longer number of animals in each group. LD_{50} is expressed in term of mg/kg. The maximum no-lethal dose was found to be 5000mg/kg body weight; hence 1/10th of the dose was taken as effective dose (500mg/kg body weight) for the different extracts of *Spilanthes acmella* leaves for diuretic activity.

Evaluation of diuretic activity

Treatment

Albino Wistar male rats (200-250g) procured from CPCSEA approved breeder (Reg. no. 126/1999/CPCSEA dated 29.6.1999)

were used for diuretic studies. Animals were kept at room temperature (26 ± 2°C) for one week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed but 18h prior to the experiment, the rats were deprived of food but water ad libitum.

Diuretic activity

The modified method of Rao was employed for the assessment of diuretic activity.[20] Male healthy Wistar albino rats (200-250g) were divided into different groups of six animals each. All the animals received priming dose of 0.9% sodium chloride solution (20 ml/kg body weight p.o.). The first group received vehicle saline (20 ml/kg body weight p.o.), served as control; the second group received the standard drug frusemide (10 mg/kg body weight p.o.), served as standard. The other groups received doses of different extracts (500 mg/kg body weight p.o.), suspended in normal saline. After oral administration, each animal was placed in an individual metabolic cage specially designed to separate faeces and urine at room temperature. The volume of urine collected was measured at the end of 5 hr and the total urine volume and concentrations of Na^+, K^+ and Cl^- in the urine were determined. The concentration of the electrolytes in urine were expressed in terms of mmol/L and the urine volume was expressed in ml/5 h. Na^+ and K^+ concentrations were measured by Flame photometer and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using 3-5 drops of 5% potassium chromate as an indicator.[21,22] The ratio of the concentration of Na^+/K^+ at the end of 5 h, were calculated to assess the diuretic potential of the different extracts of *Spilanthes acmella* leaves.

Statistical analysis

The values were expressed as mean ± SEM. The results were analyzed by using ANOVA followed by Dunnett's t-test. Statistical significance on comparison with standard drug and control groups, p values less than 0.05 were considered as significant.

RESULTS

Preliminary phytochemical results of different extracts of *Spilanthes acmella* leaves are shown in Table 1. In the present study, these tests revealed the presence of steroids only in petroleum ether extract, while chloroform extract was found to contain alkaloids, carbohydrates, tannins and steroids. Alcohol extract showed the presence of alkaloids, carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and amino acids.

Table 1: Preliminary phytochemical analysis of different extracts of *Spilanthes acmella* leaves

Chemical constituents	Petroleum ether extract	Chloroform extract	Alcohol extract
Alkaloids	-	+	+
Carbohydrates	-	+	+
Flavonoids	-	-	-
Tannins	-	+	+
Amino acids	-	-	+
Glycosides	-	-	-
Steroids	+	+	+
Sesquiterpenes	-	-	+
Carotenoids	-	-	+

+ = Positive, - = Negative

The results of different diuretic parameters are shown in Table 2. Frusemide treated animals significantly ($p < 0.01$) increased the urinary output (by 269%) and electrolyte excretion of Na^+ (by 152%), K^+ (by 185%) and Cl^- (by 136%) as compared to control. Alcohol extract significantly ($p < 0.01$) increased the urinary output (by 229%) and electrolytic excretion of Na^+ (by 135%) and K+ (by 172%), without significant renal excretion of Cl^- as compared to control. Chloroform extract also showed good diuretic action ($p < 0.05$). The diuretic action of petroleum ether extract was not significant.

dThe observed Na^+/K^+ ratio for frusemide, chloroform extract and alcohol extract were 1.41, 1.51 and 1.34 respectively, as compared to 1.70 for control. The present result shows significant diuretic potency and their effect on electrolyte excretion of different extracts of *Spilanthes acmella* comparable to the standard drug frusemide.

Table 2: Effect of oral administration of different extracts of *Spilanthes acmella* leaves on urinary volume and electrolytic excretion

Groups	Dose	Total Urine volume(ml)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Na^+/K^+
Control	20 ml/kg	5.2±0.48	80.32±3.71	47±2.92	107.78±6.62	1.70
Standard	10mg/kg	14.0±0.87**	122.83±4.63**	87±4.75**	147±6.13**	1.41
Petroleum ether extract	500mg/kg	4.9±0.61	72.10±5.21	52.89±3.74	109±6.81	1.36
Chloroform extract	500mg/kg	6.8±0.43 *	103.00±6.23 *	68.20±5.63 *	137±6.37 *	1.51
Alcohol extract	500mg/kg	11.9±0.71**	108.78±4.57**	81.0±5.46**	127.34±4.52**	1.34

Values are expressed as mean ± SEM (n = 6); *p < 0.05 and **p<0.01 compared with control (ANOVA followed by Dunnett's t-test).

DISCUSSION

The diuretic action of different extracts was evaluated using frusemide which is a high-ceiling loop diuretic, under controlled laboratory conditions. As diuretic therapy may lead to number of life-threatening electrolytic disorders and toxicities, so safety profile studies was carried out following a sub chronic administration of extracts. Results showed that there was absence of mortality and overt signs of toxicity.

This would amplify the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases.[23] the results of the present study revealed that alcohol extract induced diuresis was strong and accompanied with high natriuresis, chloruresis, and kaliuresis ($p < 0.01$). Further there was low Na^+/ K^+ ratio, so the alcohol extract seem to be acting like loop diuretics which inhibits Na^+, K^+ and Cl^- co-transport at thick ascending loop of Henle. K^+ excretion was increased perhaps due to high Na^+ load reaching the distal tube. However, chloroform extract induced both marked natriuresis and kaliuresis ($p < 0.05$), but the Na^+/ K^+ ratio was more than that of frusemide, indicating the weak kaliuresis or K^+ saving property of chloroform extract.[24] The preliminary phytochemical analysis revealed that alkaloids, carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and amino-acids are present in different extracts. These natural products

might be acting individually or synergistically to produce diuresis. It is also possible that the alcohol extract might manifest cumulative effect of several active principles in the extract.[25] These findings suggest the possible traditional use of this plant in hypertension as diuretics are used in the management of hypertension.

CONCLUSION

In conclusion, different extracts of *Spilanthes acmella* have diuretic effect supporting the ethnopharmacological use as diuretics and our results have shown that the extracts administered at the dose of 500 mg/kg body weight (p.o.) have significant effects on urinary excretion of electrolytes and support the claims of diuretic efficacy of the title plant. The present study also provides basis for the traditional use of *Spilanthes acmella* in hypertension.

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