DIURETIC EFFECT OF ETHANOL EXTRACT OF *STEREOSPERMUM SUAVEOLENS*

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Summary

The diuretic effect and the effect on urine electrolytes of ethanol extract of the bark of *Stereospermum suaveolens* (Roxb.) DC at doses of 200 and 400 mg/kg, orally, in rats were assessed using Furosemide (5 mg/kg) as a standard drug. The urine volume, pH and the concentration of ions as Na^+, K^+, Ca^{2+} and Cl^- in urine, were determined. After the administration of the ethanol extract of *Stereospermum suaveolens* (EESS), the urine volumes (ml) at the 5^{th} and 24^{th} h, were noted. The result was (1.13 ± 0.11 and 2.20 ± 0.26) for 200 mg/kg and, (2.33 ± 0.13 and 3.93 ± 0.15) for 400 mg/kg, respectively. A dose dependent diuretic activity of 1.10 and 1.04, and, 1.93 and 1.82 were observed at doses of 200 and 400 mg/kg of the extract at 5 h and 24 h duration, respectively. The electrolyte excretion was also significantly affected by the extract. The extract at both the doses significantly ($P<0.05$, $P<0.01$) increased the Na^+, Cl^- and Ca^{2+} ions accompanied by the excretion of K^+ against the control. The change in pH of urine was negligible. From the acute toxicity studies, it was observed that the EESS did not produce any mortality up to a maximum dose of 3200 mg/kg, b.w, hence the drug is found to be safe to use. These findings suggest that the ethanol extract of *Stereospermum suaveolens* possess potential diuretic activity in a dose dependent manner.

Keywords: *Stereospermum suaveolens*, Bignoniaceae, Diuretic activity, elecrolyte excretion, urine P^H, urine volume.

Introduction

Diuretics are drugs that increase the excretion of Na^+ and water from the body by the action on kidneys. Their major effect is to decrease the reabsorption of Na^+ and Cl^- from the glomerular filtrate and increase the water loss [1]. These are agents important in the therapy of cardiovascular diseases, disorders of fluid and electrolyte balance, nephritic syndrome, renal failure, cirrhosis, and pathological conditions of the kidney itself [2].

A large number of Indigenous drugs have been claimed to have a diuretic effect in the Indian system of medicine. It was found that the flowers of *Viola odorata, Nymphaea alba, Nelumbium speciosum*, roots of *Asparagus, Arundokara, Portulace oleracea* possess diuretic effect in different animal model [3,4]. Still a considerable number of plants, which possesses the diuretic activity, is yet to be explored.

Stereospermum suaveolens (Roxb.) DC, family Bignoniaceae, popularly known as padhri, is a large deciduous tree found throughout the moist parts of India. The various parts of the plant are used in native medicine. In folklore medicine, the decoction of root and bark is used as a diuretic [5]. In southern India, the bark is used in folk medicine for the treatment of diabetes pain, fever, inflammations and, asthma [5, 6]. The flowers are mixed with honey and given orally, for the control of hiccup [7]. The fruit is useful for the treatment of leprosy [8]. The root extract is known to possess anticancer activity [5,9]. The previous phytochemical studies reported the presence of lapachol, dehydro-α-lapachone [10], sterekunthal B, stereochenols A and B [11, 12] in the bark, and scutellarein [13], stereolensin [14], dinatin (4,5,7-trihydroxyl-6-methoxyflavon), and dinatin-7-glucuroniside [15] in the leaves. Literature survey reveals that the plant extract has been least screened for its traditional diuretic activity in experimental animals. Therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of *Stereospermum suaveolens* as a diuretic agent.

Materials and methods

Plant material

The bark of *Stereospermum suavelolens* was collected during October 2006 from Palode forest, Thiruvananthapuram district, Kerala, India. The plant was identified and authenticated by Dr. N. Mohanan, Scientist, the Tropical Botanical Garden and Research Institute, Palode, Tiruvananthapuram district, Kerala and a voucher specimen (TBS-1) has been deposited in our laboratory for further reference.

The bark of the plant was shade dried and powdered with a mechanical grinder. The powdered plant material was then passed through sieve No # 40 and stored in an airtight container for future use.

Preparation of plant extract

The shade dried coarse powder bark of *Stereospermum suavelolens* (500 g) was packed in the Soxhlet apparatus and extracted with 1.5 L of 95% ethanol at temperature of 40-50°C for 72 h**.** The extract was filtered and the filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at temperature of 40°C. The resultant black color residue was stored in a desiccator for use in subsequent experiments and considered as the crude ethanol extract. The yield of the extract was 11% w/w.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out to detect the chemical constituents of EESS [16,17,18].

Animals

Male Wistar albino rats (150-200 g) and female Swiss albino mice (20-25 g) were purchased from M/S.BN Ghose and Co Ltd., Kolkata, India. The animals were grouped and housed in poly acrylic cages (38 x 23 x 10 cm) with not more than 6 animals per cage and maintained under standard laboratory conditions (Temp 25 ± 2°C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for 1 week before commencement of experiment. Ethical clearance was obtained from Jadavpur University Animals Ethical Committee for using animals in the present study.

Acute oral toxicity study

Acute oral toxicity study was performed as per OECD-423 guidelines [19]. Female Swiss albino mice (20-25 g) were randomly distributed to 6 groups (n = 6). The animals were fasted overnight and the drug was administered orally (p.o) at the doses of 100, 200, 400, 800, 1600, and 3200 mg/kg b.w. The animals were closely observed for the first 24 h for any toxic symptoms and for 72 h for any mortality rate.

Evaluation of diuretic activity

Experimental Design

The method described by Lipschtiz *et al*., [20] was employed for the evaluation of diuretic activity.

Male healthy Wistar albino rats (150-200g) were divided in to five groups of six animals each and were deprived of food and water for 24 h, prior to the experiment. All the animals received a priming dose of 0.9% sodium chloride solution (25 ml/kg b.w) orally (p.o) and are treated as follows:

Group-I (Control): Normal Saline (5 ml/kg).

Group-II: Urea (1000 mg/kg, p.o).

Group-III: Standard drug (Frusemide (5 mg/kg, p.o))

Group-IV: EESS (200 mg/kg, p.o).

Group-V: EESS (400 mg/kg, p.o).

Immediately after dosing, the rats were placed in metabolic cages with special provision to separate urine and faecal matter and kept at room temperature throughout the experiment. The volume of urine was measured after oral administration of the test samples at time intervals of 5h and 24 h, respectively. During the period of study, no food and water was made available to the animals. Urine samples, which were collected from metallic cages, were analyzed for Na^+, K^+ and Ca^{2+} concentrations in mMoL/L by flame photometric method (Chemito 1020 model) [21, 22] and Cl^- concentration was determined by the titration method [23]. The pH of the urine sample (1% solution) was analysed with pH meter (Mettler Toledo, Seven Easy) [24].

The volume of urine excreted after 5 and 24h of study by control, urea, frusemide and EESS (200 and 400mg/kg) was expressed as, percent of the liquid administered giving rise to a measure of Urinary Excretion" (U.E)-independent of group weight [25],

thus

Urinary Excretion = Total urinary output/ Total liquid administration x 100

The ratio of urinary excretion in test group and control group was denoted. Diuretic action, which was used as the measure of degree of diuresis [26].

Diuretic Action = Urinary excretion in test group/ Urinary excretion in control group

Diuretic Activity = Diuretic action of test/ Diuretic action of urea.

Results

Preliminary phytochemical analysis

The preliminary phytochemical investigation revealed that the presence of favonoids, terpenoids, saponins, alkaloids, tannins, steroids, deoxy sugars and gums in the ethanol extract.

Acute oral toxicity study

The ethanol extract of *Stereospermum suaveolens* does not produced any mortality up to 3200 mg/kg b.w. Further dosing was not performed to estimate the LD_{50} (lethal dose) value. According to the OECD guidelines for the acute toxicity, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe.

Urine output and diuretic activity

The urinary output over a period of 5 h and 24 h of study has been presented in table1 and 2. The urine volume was increased significantly in EESS of both doses, urea and frusemide administered rats compared with control rats. The EESS 200 mg/kg increased the urine volume of 1.30 ± 0.11 and 2.20 ± 0.26, and 400 mg/kg of 2.33 ± 0.13 and 3.93 ± 0.15 at 5 h and 24 h, respectively. Control, urea and frusemide showed 0.66 ± 0.04 and 1.23 ± 0.08, 1.10 ± 0.06 and 1.96 ± 0.14, and 2.40 ± 0.08 and 4.06 ± 0.10, respectively Followed by the measure of urine volume, diuretic activity of frusemide and EESS (200 and 400 mg/kg) were calculated, which showed the extracts act in a dose dependent manner.

Electrolytes excretion and pH

The electrolytes excretion potency of the extract was highly moderate in comparison with control animals (Table.1 and 2). EESS (200 mg/kg) treated animals significantly increased the electrolyte excretion of Na^+ (196.16 ± 7.52 and 177.83 ± 4.90), K^+ (92.16 ± 3.49 and 89.33 ± 5.23), Ca^{2+} (249.66 ± 6.60 and 228.16 ± 4.70) and Cl^- (242.50 ± 3.99 and 201.16 ± 7.24) as compared to control group after 5 h and 24 h, respectively. The highest dose of EESS (400 mg/kg) enhanced significantly, the urine excretion of Na^+ (223.16 ± 6.90 and 194.83 ± 6.25), K^+ (115.50 ± 3.89 and 108.83 ± 3.79), Ca^{2+} (316.83 ± 7.42 and 263.50 ± 3.47) and Cl^- (264.66 ± 3.53 and 238.50 ± 9.76) as compared to control group. Frusemide treated animals significantly ($P<0.01$) increased the electrolyte excretion of Na^+ (224.35 ± 0.69 and 211.83 ± 6.02), K^+ (110.33 ± 3.77 and 120.00 ± 8.72), Ca^{2+} (272.33 ± 6.73 and 259.83 ± 5.11) and Cl^- (280.50 ± 7.15 and 252.00 ± 5.89). The observed Na^+ / K^+ ratio for frusemide, EESS 200 mg/kg and EESS 400 mg/kg were 2.03 and 1.75, 2.12 and 1.99, and 1.93 and 1.79, respectively, as compared to (2.18 and 2.19) control. The pH of urine (1% solution) was alkaline in nature.

Table 1. Diuretic effect of ethanol extract of *Stereopsermum suaveolens* after 5 h by oral administration.

Groups	Urine Volume (ml)	Urinary Excretion	Diuretic Action	Diuretic Activity	Urine pH	Electrolytes excretion (mM/L)				
						Na^+	K^+	Ca^{2+}	Cl^-	Na^+/K^+
Control Saline (5ml//kg)	0.66±0.04	19.35	-	-	6.83±0.24	166.16±6.29	76.16±2.33	126.33±3.99	159.00±2.75	2.18
Urea (1000mg/kg)	1.10±0.06*	35.48	1.83	-	7.06±0.31	196.50±5.48*	90.66±3.29*	152.50±5.59*	198.33±9.44*	2.16
Frusemide (5mg/kg)	2.40±0.08**	80.00	4.13	2.25	7.24±0.35	224.35±0.69**	110.33±3.77**	272.33±6.73**	280.50±7.15**	2.03
EESS (200mg/kg)	1.3±0.11**	3.80	2.00	1.10	7.28±0.40	196.16±7.52*	92.16±3.49*	249.66±6.60**	242.50±3.99**	2.12
EESS (400mg/kg)	2.33±0.13**	68.33	3.53	1.93	7.33±0.29	223.16±6.9**	115.50±3.89**	316.83±7.42**	264.66±3.53**	1.93

Values are expresses as mean ± S.E.M, n=6. The statistical significance was preformed by ANOVA followed by Dunnet's test, where **P <0.01, *P<0.05 in experimental groups when compared with control.

Table 2. Diuretic effect of ethanol extract of *Stereopsermum suaveolens* after 24 h by oral administration.

Groups	Urine Volume (ml)	Urinary Excretion	Diuretic Action	Diuretic Activity	Urine pH	Electrolytes excretion (mM/L)				
						Na^+	K^+	Ca^{2+}	Cl^-	Na^+/K^+
Control Saline (5ml//kg)	1.23±0.08	36.07	-	-	7.20 ±0.27	136.83 ±3.26	62.38 ± 2.51	143.96 ± 5.36	130.16±6.49	2.19
Urea (1000mg/kg)	1.96±0.14*	63.23	1.75	-	7.38±0.36	160.16 ±5.35*	84.83 ±3.51*	166.00 ±4.31*	186.16±4.88**	1.88
Frusemide (5mg/kg)	4.06±0.10**	135.33	3.75	2.14	7.75±0.40	211.83±6.02**	120.00±8.72**	259.83±5.11**	252.00±5.89**	1.75
EESS (200mg/kg)	2.20±0.26*	65.67	1.8	1.04	7.43±0.50	177.83±4.90**	89.33±5.23*	228.16±4.70**	201.16±7.24**	1.99
EESS (400mg/kg)	3.93±0.15**	115.24	3.19	1.82	7.65±0.4	194.83±6.25**	108.83±3.79**	263.50±3.47**	238.50±9.76**	1.79

Values are expresses as mean ± S.E.M, n=6. The statistical significance was preformed by ANOVA followed by Dunnet's test, where $^{**}P < 0.01$, $^*P<0.05$ in experimental groups when compared with control.

Discussion

Diuresis has two separate functions, one is to increase the urine volume per sec and the other is net loss of electrolytes and water [27,28]. These Two processes are involved in the suppression of renal tubular reabsorption of electrolytes and water and low molecular weight organic compounds in to the blood stream and as a consequence, promoting the formation of urine and eliminating toxic substances [27,29]. Since, the plant *Stereospermum suaveolens* has traditional use as a diuretic, the effect of ethanol extract of the plant on urination and excretion of electrolytes were investigated in male Wistar rats.

In the present work, the ethanol extract of *Stereospermum suaveolens* was found to exhibit significant and dose dependent increase in urine volume and also the excretion of Na^+, K^+, Ca^{2+} and Cl^- ions in the urine of treated rats at both the of doses (200 and 400 mg/kg, p.o.). The results revealed that the effect of the extract is similar to that of frusemide.

The diuretic activity of the extract is considered to be good, if it is above 1.5, moderate if it is within 1.0-1.5, little if it is between 0.7-1.0, and nil if it is less than 0.72 [26]. The report of the study reveals that, the EESS was found to produce dose dependent moderate (1.10 and 1.04) and good (1.93 and 1.82) diuretic activities at doses of 200 and 400 mg/kg after 5 h and 24 h, respectively. Flavonoids, saponins, sterols and triterpenes [30, 31] are known to possess diuretic activity. Flavonoids, terpenoids, saponins and steroids were found to be present in the extract during phytochemical analysis, they may be responsible for the diuretic activity.

The mechanism of action by which diuresis was induced by the extract, was also investigated by comparing the activity of the extract with that of a standard drug as frusemide [32,33]. Frusemide act primarily on the thick ascending loop of Henle and inhibits Na^+–K^+–$2Cl^-$ contransport [34].

The EESS induced diuresis was good and accompanied with high natriuresis, chloruresis, calciuresis and kaliuresis. Further, there was low Na^+ / K^+ ratio; so the EESS seem to be acting like loop high ceiling diuretics (Frusemide) which inhibits Na^+, K^+, Ca^{2+} and Cl^- co-transport at thick ascending loop of Henle and resulting in the elimination of water. K^+ excretion was increased perhaps due to high Na^+ load reaching the distal convoluted tube [35].

After 5 h of the EESS administration an increase in urine output at the both dose levels were observed and the effect is slightly reduced after 24 h.

Both doses of the extracts increased the electrolyte excretion of sodium, calcium and chloride accompanying with potassium when compared with control after 5 h and 24 h. It showed the extract acts in a time and dose dependent manner. In all the cases the excretion of electrolytes increased was more or less similar to the standard drug frusemide.

The above results suggest that the ethanol extracts of *Stereospermum suaveolens* is an effective hypernatraemic, hyperchloremic, hyperkalemic and hypercalcemic diuretic which supports the traditional claim of the plant being used as a diuretic.

Conclusion

The present study emphasizes that the ethanol extract of SS bark exerted dose dependent diuretic activity and the real mode of action is not evident from the experimental result. Further studies are necessary to identify the active constituent(s) and investigate the molecular mechanism of diuretic effect.

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