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Evaluation of diuretic, saluretic, natriuretic and carbonic anhydrase inhibition activity of hydro-alcoholic extract of seeds of *Paspalum scrobiculatum* Linn. (poaceae) in Wistar albino rats

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ABSTRACT

The purpose of this research work is to determine the diuretic, saluretic, natriuretic, and carbonic anhydrase inhibition activity of hydroalcoholic extract of seeds of *Paspalum scrobiculatum* Linn in Wistar albino rats. Wistar albino rats of either sex were divided into four groups. The control group received 0.1 mg/ml of CMC, the reference group receive frusemide (10 mg/kg) and the test group received 200 mg/kg and 400 mg/kg of hydroalcoholic extract of *Paspalum scrobiculatum* by oral route. The parameters measured for diuretic activity were urine volume and urinary concentration of electrolytes such as sodium, potassium and chloride. The crude hydroalcoholic extract of the plant at the dose 200 mg/kg and 400 mg/kg showed significant diuretic ($p<0.01$ and $p<0.0001$ respectively), saluretic ($p<0.0001$), and natriuretic activity ($p<0.01$ and $p<0.0001$ respectively). The diuretic index of the test group (200 mg/kg and 400 mg/kg) was 1.6822 ± 0.163 and 2.292 ± 0.1690 respectively. The present data about various parameters like urinary volume and electrolyte concentration (Na^+, K^+, and Cl^-) indicate that *Paspalum scrobiculatum* has an ideal diuretic and natriuretic activity.

INTRODUCTION

The drug that is used to enhance the urine volume is termed diuretics [1]. Diuretics play a critical function in conditions of fluid overload, like cirrhosis of the liver, acute and chronic renal failure, hypercalciuria, and additionally as an antihypertensive agent. Diuretics relieve pulmonary congestion and peripheral oedema. These agents are beneficial in decreasing the syndrome of volume overload, consisting of orthopnea and paroxysmal nocturnal dyspnoea. They lower plasma volume and finally venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus lowering blood pressure. The currently available diuretic agents are often associated with many adverse reactions [2,3]. Thiazide-like diuretics produce side effects that include decreased libido, erectile disfunction and difficult ejaculation [4].

Paspalum scrobiculatum Linn. belonging to the family Poaceae, is identified as the true botanical source of Kodrava. It is commonly known as 'Kodo millet', which is a tufted perennial grass, up to 120-150 cm tall, culms stout glabrous, somewhat bulbous at the base. It is found across the old world in humid habitats of tropics and subtropics. It is a minor grain crop in India, and an important crop in the Deccan plateau. Its cultivation in India is generally confined to Gujarat, Karnataka, Chhattisgarh, Eastern Madhya Pradesh, and Tamil Nadu. Several medicinal properties such as antidiabetic, tranquilising, antirheumatic and wound-healing are attributed to this grain. Traditionally it is used in diabetes, hypertension, depression, wound healing, cancer, hyperlipidaemia, microbial infections, inflammation, haemorrhages, and general debility. According to the phytochemical investigation, the seeds of *Paspalum scrobiculatum,* contain tannins, phenolic compounds, terpenoids, glycosides, amino acids, fixed oil and fat, saponins, flavonoids,

protein and carbohydrates [5].

Therefore, there may be well-timed want of discovering efficacious tremendously cheap novel diuretic with less adverse effects presumably from the natural source. Accordingly, this systematic study was carried out to evaluate the diuretic, saluretic, natriuretic and carbonic anhydrase inhibition activity of hydro-alcoholic extract of seeds of *Paspalum scrobiculatum* Linn. in Wistar albino rats.

MATERIALS AND METHODS

Collection and authentication of plants

The seeds of *Paspalum scrobiculatum* were collected from the Mana velugu Vintage farmers, Andhra Pradesh. Plants were cultivated and harvested in Oruvathilkotta, Trivandrum. The plant materials used for the study were authenticated by Dr T.S. Swapna, Professor and Head of the department of botany, University of Kerala, Kariavattom, Thiruvananthapuram. The herbarium of plant materials was prepared and submitted to the University of Kerala, Kariavattom (voucher specimen number: KUBH 10477).

Preparation of extracts

Seeds were washed with water and dried in shade to remove the traces of moisture. The seeds were powdered for the easiness of extraction and stored in an airtight container. The dry powdered plant material was sieved through sieve No: 60. Then the coarse powder was collected. First, the powdered seeds were subjected to extraction with petroleum ether (60-80^{0}C) by using the Soxhlet apparatus for the removal of fatty materials. Then defatted plant material was dried at room temperature and finally subjected for Soxhlation with 70 % ethanol. A total of 250 g of plant material was used for hydro-alcoholic extraction. After successive soxhlation for 24 hours, the extract was distilled to remove the solvent completely and dried in a desiccator. Finally, the extract was weighed and calculated the percentage yield [6].

Experimental animals

Wistar albino rats (150 350 g) of both sexes were used for this study. The animals were housed under the standard conditions of temperature (25 ± 2 ^{0}C) and relative humidity (33 70%) with a 12:12 light-dark cycle. The animals were fed with a standard rodent pellet diet and water ad libitum except during experimentation. Experiments were conducted as per the CPCSEA guidelines. The Institutional Animal Ethics Committee approval number was 02/05/2020/IAEC/MCT.

Chemicals

Furosemide (Sigma Aldrich, USA was used as the reference drug). CMC (Central drug house, India), $AgNO_3$ (Central drug house, India) and NaCl (Central drug house, India)

Diuretic activity

Lipschitz Test

Male Wistar rats weighing 150-350 g were used for the study. They were marked individually for identification. The animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless steel sieves were placed in the funnel to retain faeces and to allow the urine to pass. The rats were fed with a standard diet and water ad libitum. Fifteen hours before the experiment, food and water were withdrawn. 200 and 400 mg/kg of the test compound *Paspalum scrobiculatum* were administered orally. The positive control group animals received 10 mg/kg frusemide orally. Additionally, 5 ml of 0.9 % NaCl solution per 100 g body weight were given by gavage. Urine excretion was recorded after 5 and after 24 h [7,8,9].

Urine volume excreted per 100 g body weight was calculated for each group. Results were expressed as the Lipschitz value ie, the ratio T/U.

T= The response of the test compound

U= The response of the frusemide treatment

$$Diureic\ index = \frac{urine\ volume\ of\ the\ sample}{urine\ volume\ of\ the\ control}$$

$$Diuretic\ activity = \frac{diuretic\ index\ of\ the\ test}{diuretic\ index\ of\ the\ standard}$$

Saluretic activity in Rats

Male Wistar rats weighing 150-350 g fed with a standard diet (Altromin® pellets) and water ad libitum were used. Fifteen hours before the test, food but not water was withdrawn. One animal was placed in one metabolic cage provided with a wire mesh bottom and a funnel to collect the urine. Six animals were used for each dose of a test drug. The test compound *Paspalum scrobiculatum* at the doses 200 and 400 mg/kg were administered orally. Urine excretion was registered every hour up to 5 h. The 5 h urine was analysed by flame photometry for sodium and potassium. The chloride was analysed through endpoint titration argentometrically. To evaluate compounds with prolonged effects, the 24 h urine was collected and analysed. Furosemide (10 mg/kg p.o.), was used as standard. The sum of sodium and chloride were calculated as parameters for Saluretic activity. The ratio of sodium and potassium was calculated for natriuretic activity. The ratio of Cl^- / ($Na^+ + K^+$) was calculated to estimate carbonic anhydrase inhibition [10,11,12].

$$Saluretic\ index = \frac{concentration\ of\ electrolyte\ in\ urine\ of\ test\ group}{concentration\ of\ electrolyte\ in\ urine\ of\ control\ group}$$

$$Saluretic\ activity = Na^+ + Cl^-$$

$$Natriuretic\ index = \frac{urinary\ excretion\ of\ Na^+}{urinary\ excretion\ of\ K^+}$$

$$Carbonic\ anhydrase\ inhibition = \frac{Cl^-}{Na^+ + K^+}$$

Statistical analysis

The results were expressed as mean ± SEM, data were determined using one way ANOVA followed by Dunnett's

multiple comparison.

RESULTS

Lipschitz Test

In the Lipschitz method, the urine volume of the low dose and high dose of the plant extract after 5 h of administration were 1.704 ± 0.1624 ($p<0.01$) and 2.323 ± 0.1713 ($p<0.0001$) respectively. This study showed a significant increase in the urine output produced by the plant extract as compared with the control group which showed the urine output 1.019 ± 0.0340. The diuretic index and diuretic activity produced by low dose plant extract were 1.6822 ± 0.163 and 0.6404 and for high dose, it was 2.292 ± 0.1690 and 0.8729 respectively (Table 1).

Table 1 : Diuretic activity of hydroalcoholic extract of seeds of *Paspalum scrobiculatum* after 5 hours of administration

Group	Urine volume (ml/100g/5h)	Urinary Excretion (mEq/kg)			Diuretic Index	Lipschitz value
		Na^+	K^+	Cl^-		
control	1.013 ± 0.0340	39.166 ± 2.651	37.761± 1.928	78 ± 0.410	-	-
Paspalum scrobiculatum 200 mg/kg	1.704 ± 0.1624 **	102.752± 7.978 ****	66.58 ± 3.096 ****	142.216 ± 1.478 ****	1.6822 ± 0.163 **	0.6404
Paspalum scrobiculatum 400 mg/kg	2.323 ± 0.1713 ****	129.180± 0.8820 ****	52.80 ± 1.1026 *	154.23 ± 1.1627 ****	2.292 ± 0.1690	0.8729
Frusemide 10 mg/kg	2.662 ± 0.1191 ****	151.25 ± 3.461 ****	97.72 ± 1.302 ****	207.28 ± 1.603 ****	2.626 ± 0.1965	-

All values are Mean ± SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test, *$p<0.05$, **$p<0.01$, and ****$p<0.0001$ when compared with the control.

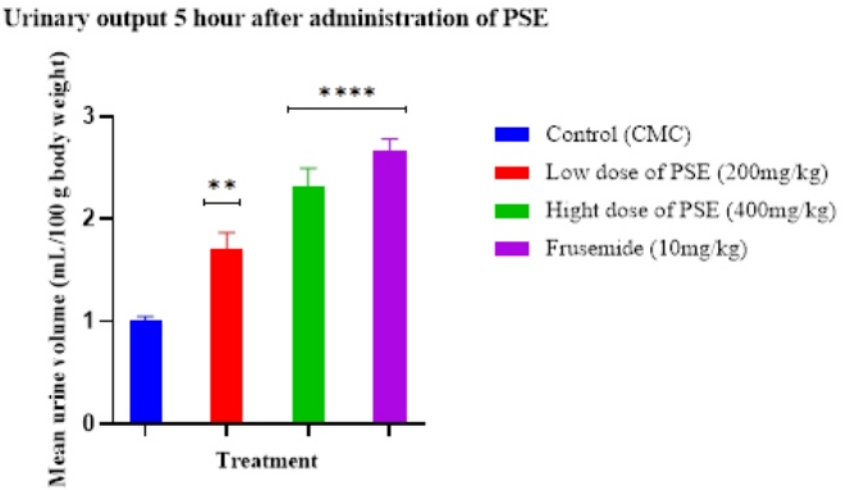

Fig 1 : *Comparison of urinary output 5 h after administration of the drug.* Values are Mean ± SEM, N = 6, **$p<0.01$ and ****$p<0.0001$ when compared with the control.

The urine volume of the low dose and high dose of the plant extract after 24 h of administration were 0.9497 ± 0.0557 and 0.9474 ± 0.0485 respectively. The diuretic index and diuretic activity produced by the low dose of plant extract were 1.343 ± 0.0698 and 0.8688 and for high dose, it was 1.1878 ± 0.0609 and 0.9098 respectively (Table 2).

Table 2 : Diuretic activity of hydroalcoholic extract of seeds of *Paspalum scrobiculatum* after 24 hours of administration

Group	Urine volume (ml/100g/5h)	Urinary Excretion (mEq/kg)			Diuretic Index	Lipschitz value
		Na^+	K^+	Cl^-		
control	0.7976 ± 0.0636	39.716 ± 1.4510	34.333 ± 1.8626	71.258 ± 1.8391	-	-
Paspalum scrobiculatum 200 mg/kg	0.9497 ± 0.0557	44.798 ± 2.2160	41.386 ± 0.4834	78.466 ± 0.3303	1.1343 ± 0.0698	0.8688
Paspalum scrobiculatum 400 mg/kg	0.9474 ± 0.0485	45.110 ± 2.2068	40.980 ± 0.6432	77.916 ± 0.3297	1.1878 ± 0.0609	0.9098
Frusemide 10 mg/kg	1.0412 ± 0.0876	43.826 ± 2.5475	41.361 ± 0.5310	76.470 ± 1.7227	1.0412 ± 0.0876	-

All value are Mean ± SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test.

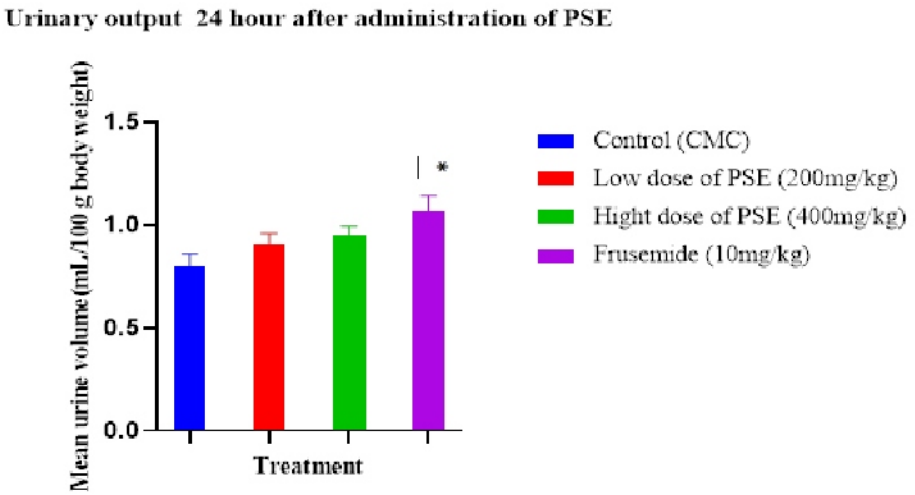

Fig 2 : Comparison of urinary output 24 h after administration of the drug. Values are Mean + SEM, N = 6, *p<0.05 when compared with the control.

Saluretic activity in rats

The sodium excreted by the low and high dose of plant extract after 5 h after administration were 102.72 ± 7.978 ($p<0.0001$) and 129.18 ± 0.8820 ($p<0.0001$) respectively. This study showed a significant increase in the sodium excretion produced by the plant extract as compared with the control group (1.019 ± 0.0340). The potassium excretion produced by the low dose and high dose of plant extract was 66.58 ± 3.096 ($p<0.0001$) and 52.80 ± 1.1026 ($p<0.05$) respectively. This study showed a decrease in potassium excretion with an increase in the concentration of plant extract when compared with the control group (37.716 ± 1.928). The chloride excretion produced by the low dose and high dose of plant extract was 142.216 ± 1.478 ($p<0.0001$) and 154.23 ± 1.1627 ($p<0.0001$) respectively. This study showed a significant increase in the chloride excretion produced by the plant extract as compared with the control group which showed the chloride excretion 78 ± 0.410 (Table 1).

After 24 h of the administration of plant extract, sodium excretion produced by the low dose and high dose of plant extract were 44.798 ± 2.2160 and 45.110 ± 2.2068 respectively. This study showed that the sodium excretion produced by the plant extract was not significant compared with the control group which showed sodium excretion 39.716 ± 1.4510. The potassium excretion produced by the low dose and high dose of plant extract were 41.386 ± 0.4834 and 40.980 ± 0.6432 respectively. And it was non-significant compared with the control group which showed potassium excretion 34.333 ± 1.8626. The chloride excretion produced by the low dose and high dose of plant extract were 78.466 ± 0.3303 and 77.916 ± 0.3297 respectively. And it was also non-significant compared with the control group which showed the chloride excretion of 71.258 ± 1.8391 (Table 2).

The saluretic activity produced by the low dose and high dose of the plant extract after 5 hours were 169.33 ± 9.792 ($p>0.0001$) and 181.98 ± 1.692 ($p>0.0001$) respectively (Table 3). There was a significant increase in the saluretic activity produced by the plant extract as compared with the control group (80.961 ± 3.076). The saluretic activity of plant extract indicates a positive effect after 5 hours of administration. But not effective after 24 hours of administration.

The natriuretic activity produced by the low dose and high dose of the plant extract after 5 hours were 1.5479 ± 0.0990 ($p<0.0001$) and 2.4512 ± 0.0496 ($p<0.01$) respectively (Table 3). This study showed a significant increase in the natriuretic activity produced by the plant extract compared with the control group (1.1709 ± 0.0946). The natriuretic activity of plant extract increased in a dose-dependent manner. But it was not effective after 24 hours of administration.

DISCUSSION

This study evaluates the diuretic, saluretic, natriuretic and carbonic anhydrase inhibition activity of hydro-alcoholic extraction of seeds of *Paspalum scrobiculatu*m in Wistar albino rats. *Paspalum scrobiculatum* was selected for study since it has been used for the same purpose in traditional medicine.

In the present study, the diuretic activity is considered to be good when the diuretic index values are higher than 1.50, moderate when the values are between 1.00 to 1.50, mild when the values are from 0.72 to 1.00 and no diuretic activity exists with

Table 3 : Saluretic, Natriuretic and Carbonic anhydrase activity of hydroalcoholic extract of seeds of *Paspalum scrobiculatum* after 5 hours of administration

Group	Saluretic index			Saluretic activity	Natriuretic index	CA inhibition
	Na^+	K^+	Cl^-			
control	-	-	-	80.961 ± 3.076	1.1709 ± 0.0946	0.9704 ± 0.0355
*Paspalum scrobiculatu*m 200 mg/kg	2.62	1.76	1.82	169.33 ± 9.792 ****	1.5479 ± 0.0990 **	0.8525 ± 0.0449
*Paspalum scrobiculatu*m 400 mg/kg	3.29	1.39	1.97	181.98 ± 1.692 ****	2.4512 ± 0.0496 ****	0.8482 ± 0.0162
Frusemide 10 mg/kg	3.86	2.59	2.65	248.90 ± 3.589 ****	1.5493 ± 0.0422 **	0.8337 ± 0.0171

All values are Mean + SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test, **p<0.01 and ****p<0.0001 when compared with the control.

Table 4 : Saluretic, Natriuretic and Carbonic anhydrase activity of hydroalcoholic extract of seeds of *Paspalum scrobiculatum* after 24 hours of administration

Group	Saluretic index			Saluretic activity	Natriuretic index	CA inhibition
	Na^+	K^+	Cl^-			
control	-	-	-	74.050 ± 2.913	1.1674 ± 0.0528	0.9715 ± 0.0518
Paspalum scrobiculatum 200 mg/kg	1.12	1.20	1.10	86.185 ± 2.297	1.0830 ± 0.0551	0.9138 ± 0.0262
Paspalum scrobiculatum 400 mg/kg	1.13	1.19	1.09	86.090 ± 2.245	1.1025 ± 0.0593	0.9082 ± 0.0249
Frusemide 10 mg/kg	1.10	1.20	1.07	85.189 ± 2.345	1.0623 ± 0.0698	0.9016 ± 0.0362

All values are Mean + SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test.

In carbonic anhydrase inhibition activity, plant extract shows a non-significant effect.

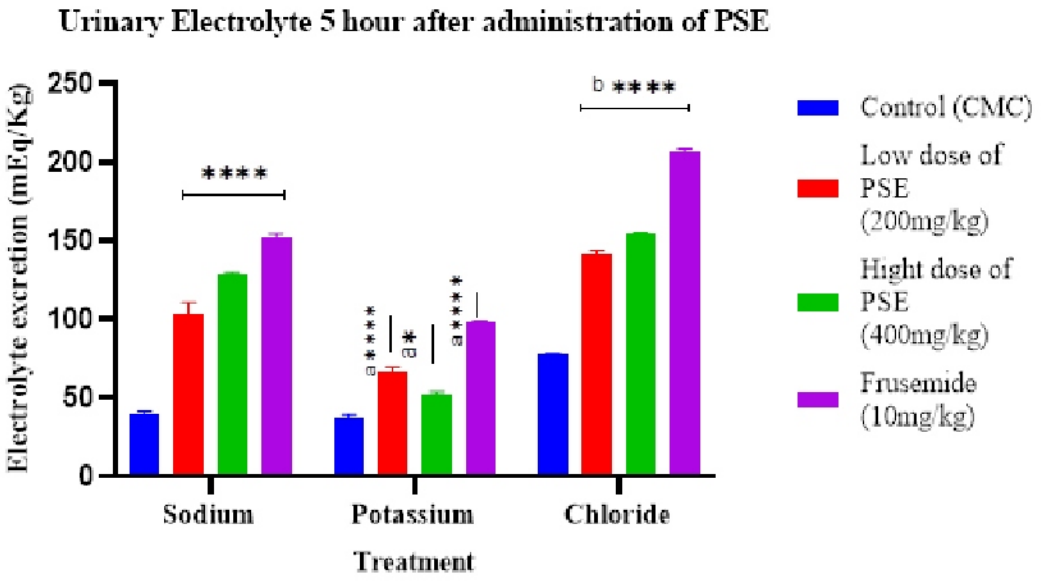

Fig 3: Comparison of urinary electrolyte 5 h after administration of plant extract. Values are Mean + SEM, N = 6, ****p<0.0001 when compared with the sodium control. a****p<0.0001 and a* p< 0.05 when compared with the potassium control. b****p<0.0001 when compared with the chloride control.

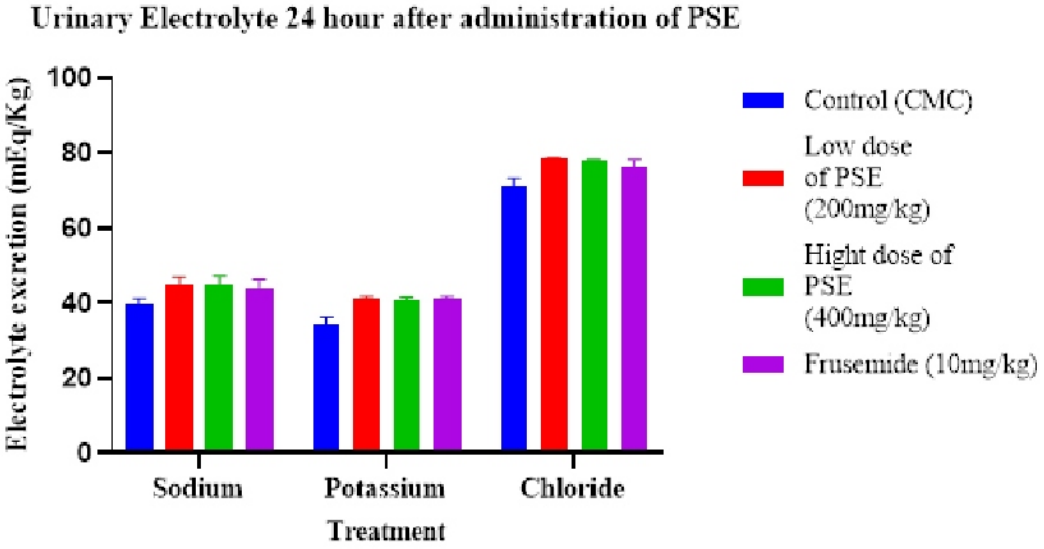

Fig 4: Comparison of urinary electrolyte 24 h after administration of plant extract. Values are Mean + SEM, N = 6.

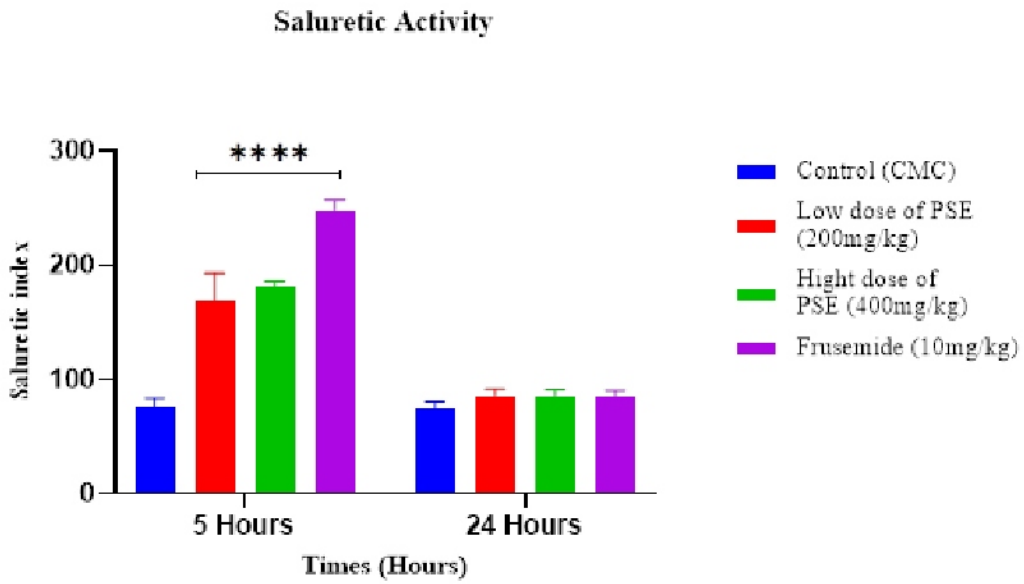

Fig 5: Comparison of the saluretic activity of plant extract. Values are Mean + SEM, N = 6, ****p<0.0001 when compared with the control.

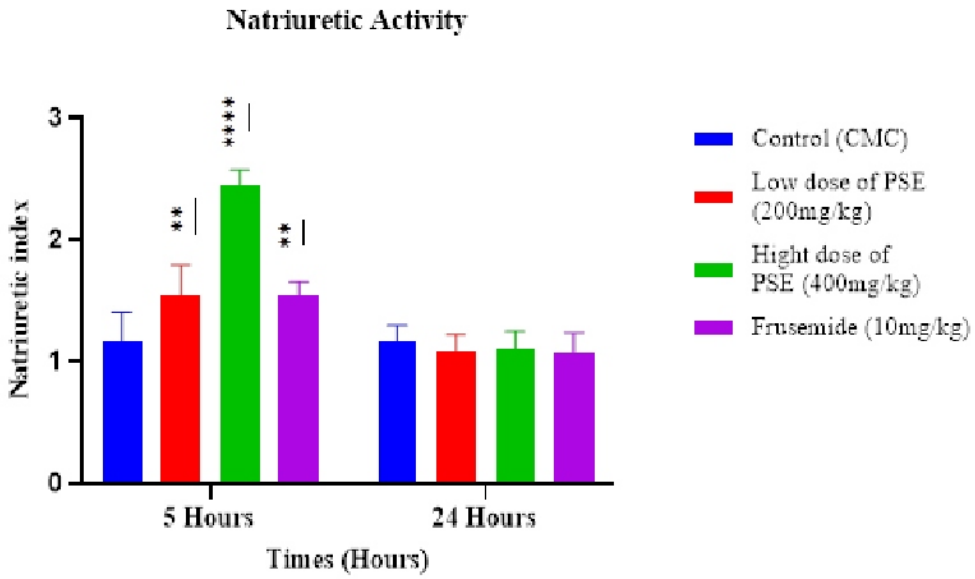

Fig 6: Comparison of the natriuretic activity of plant extract. Values are Mean + SEM, N = 6, **p<0.01 and ****p<0.0001 when compared with the control.

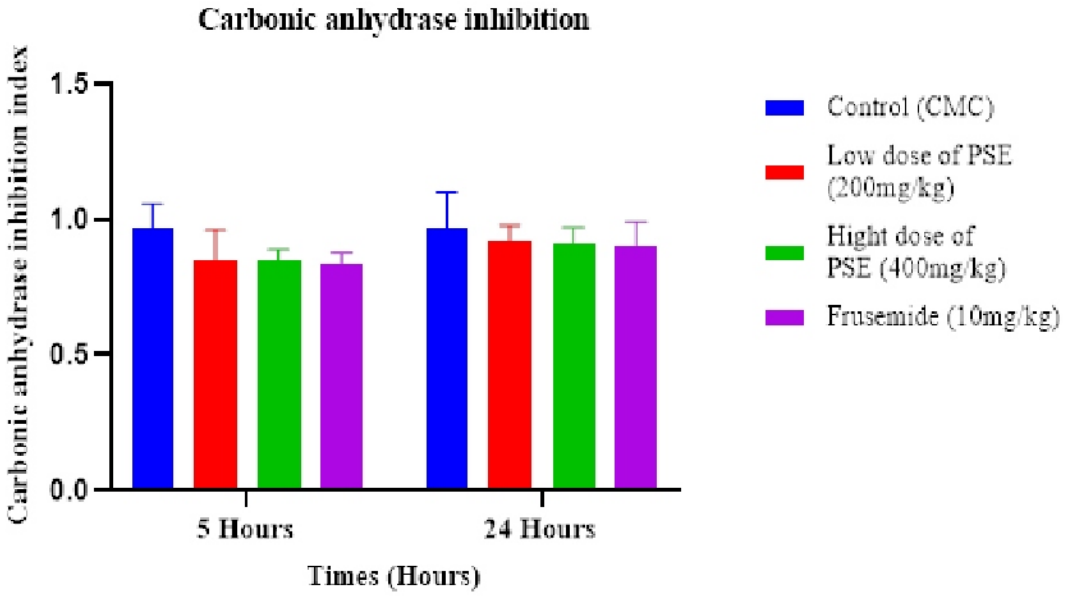

Fig 7: Comparison of carbonic anhydrase inhibition of plant extract. Values are Mean + SEM, N = 6.

values below 0.72 [11]. Both the furosemide group and *Paspalum scrobiculatu*m group showed a diuretic index value>1.50, indicating good diuretic activity.

In saluretic activity, 200 mg/kg and 400 mg/kg doses of *Paspalum scrobiculatum* produced a dose-dependent increase in the excretion of Na^+ and Cl^-, but K^+ excretion was found to be decreasing with increasing concentration of the extract. The ratio of Na^+/K^+ greater than 2 indicate natriuretic effect and ratio greater than 10 indicate potassium-sparing effect [12]. The *Paspalum scrobiculatum* 400 mg/kg group showed a ratio >2, indicating the natriuretic activity. Inhibition can be excluded at the ratio between 1 to 0.8 with decreasing ratio slight to strong carbonic anhydrase inhibition can be assumed. Since all the treatment groups had values between 0.8 and 1, carbonic anhydrase inhibition can be

ruled out.

Na^+ is considered as one of the important external factors in primary hypertension. Increased Na^+ uptake has been known to produce adverse effects on arterial blood pressure [15]. Our study showed that, the oral administration of *Paspalum scrobiculatu*m produced significant natriuretic effects especially at 200 mg/kg and 400 mg/kg compared to the 0.1 % CMC treated group. Similarly, K^+ in the urine samples significantly decreased with the increasing dose of *Paspalum scrobiculatum*. The excretion of K^+ in the treated groups was less than that of the reference group suggesting potassium-sparing properties of *Paspalum scrobiculatu*m. It is hypothesized from the observations that the diuretic action of *Paspalum scrobiculatu*m might be the outcome of inhibition of epithelial sodium channels or aldosterone action [16].

CONCLUSION

The present data about various parameters like urinary volume and electrolyte concentration (Na^+, K^+ and Cl^-) indicate that *Paspalum scrobiculatum* has an ideal diuretic and natriuretic activity. However, identification of the active principles responsible for the above activities and the mechanism of action is yet to be studied in detail.

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