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Original Research Article

Effect of *Sesbania grandiflora* Linn leaf extracts on diuresis in wistar rats

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ABSTRACT

Background: This study was designed to evaluate the effect of sesbania grandiflora linn leaf extracts on diuretic activity in wistar rats.
Methods: Healthy male wistar albino rats divided into 9 groups and weighing 150-200gm.diuretic activity was expressed by Natriuretic activity, saluretic activity, carbonic anhydrase inhibition, Diuretic index or Diuretic action, saluretic index, Natriuretic index. Animalgroups are divided into: group1 treated as control, 2and 3 treated as standards, group 4, 5 and 6 treated with aqueous leaf extract of *Sesbania grandiflora* Linn. in different doses of 150,300,450mg/kg. Group 7,8and 9 treated with methanolic leaf extract of *Sesbania grandiflora* Linn. Doses of 150,300,450mg/kg.
Results: No change in body weight group treated with AE-150 ($p>0.05$) with AE-150 (0.1%), increase in urine volume with increasing doses both with aqueous and methanolic extracts, sodium excretion with methanolic extract was high compared with the aqueous extract, significant increase in potassium excretion in both aqueous and methanolic extract treated groups.All the statistical tests were done by using SPSS version 6. The level of significance was 5% ($p\leq0.05$).
Conclusions: All the electrolytes concentration was increased in the urine, hence excluding osmotic diuretic action. Diuretic index, saluretic activity, saluretic index and natriuretic index of ME-450 were more than the value of hydrochlorothiazide hence excludes thiazide diuretic action. But these values were less than furosemide.

Keywords: Aqueous and methanolic extract, Diuresis, *Sesbania grandiflora* Linn

INTRODUCTION

Diuretics are drugs that increase the rate of urine flow.[1,2] They are used as first line drugs in treating hypertension (HTN).[3] Hypertension is considered to be a predisposing factor for stroke, coronary heart disease, and heart failure and end stage renal disease.[4] Nearly one billion people worldwide have high blood pressure and that number is expected to increase to 1.56 billion by 2025.[4] Hence controlling of HTN is most essential. Diuretics reduce the volume overload and bring relief from orthopnea and paroxysmal nocturnal dyspnea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand. Hence it is used in congestive heart failure.[5,6]

A number of diuretics like thiazides, furosemide, mannitol, and spiranolactone etc are used in practice.

Most diuretic drugs have the adverse effects such as electrolyte disturbances, acid-base disturbances, hyperuricemia, hyertriglyceridemia and hyperglycemia etc.[2,7] Although most of the diuretics proved to be very effective in promoting sodium excretion, all cause potassium loss[2] and prompted the search for potassium sparing diuretic. Hence search for a new diuretic agent that retains therapeutic efficacy and yet devoid of potassium loss is justified. Many indigenous drugs have been claimed to have diuretic effect in ayurvedic system of medicine but they were not properly investigated.[7,8]

Medicinal plants play an important role in the development of potent therapeutic agents. Ex. reserpine, vinblastine, vincristine, artemisinin and paciltaxel. The utilization of plants for medicinal purpose in India has been documented long back in ancient literature because they are essential to human survival.[9] Hence an effort has been made to evaluate the diuretic activity of leaf extracts of *Sesbania grandiflora Linn.* Despite the popular use of this medicinal plant, minimal pharmacological or clinical study was carried out to test the diuretic activity of the leaves of this plant. So this experimental study is taken to evaluate the diuretic activity of leaf extracts of *Sesbania grandiflora Linn.*

METHODS

Experimental animals

Malewistar albino rats of weighing 150-200gmswere procured from animal house of NRI Medical College, Chinakakani. The experiment was carried out in research laboratory, Department of Pharmacology, NRI Medical College after protocol was approved during Aug 2012 to Oct 2014. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 25±1°C and standard 12h:12h day/night rhythm with relative humidity. All the animals had free access to water provided in drinking bottles and fed with standard pellets.

Collection of plant materials

Aqueous leaf extract of *Sesbania grandiflora Linn and* Methanolic leaf extract of *Sesbania grandiflora Linn* obtained from the Nuzen Pvt Ltd, Hyderabad after standardization.

Experimental method

The modified method was employed for the assessment of diuretic activity.[10,11] Rats were placed in metabolic cages 24hrs prior to commencement of the study for adaptation. Rats were fasted for 18 hours before the experiment. Total duration of treatment was 7 days. In the seven days, experiment was conducted on day1 and day7. From day 2 to 6 only treatment was given without experiment.

On day1, body weight of individual rat was measured. They were hydrated with 5ml/kg of distilled water prior to drug/extract administration and then treated with respective drugs as stated above along with distilled water as solvent and then animals were transferred to metabolic cages (4 animals in a cage) specially designed to separate urine and feces. Animals were observed for 24hrs during which food and water was not provided. After 24hrs, animals were removed from the metabolic cages; total 24hrs urine volume was collected in test tubes.[12]

Na^+, K^+ and Cl^- concentrations were estimated using 24hrs urine sample. Treatment was continued for another 6 days. On day 7, same experimental procedure was repeated and 24 hrs urine volume, urinary electrolytes and post treatment body weight was measured. Temperature was maintained at 25±1°C.[13] This procedure was repeated four times (session 1 to 4) with each treatment group as stated below and above parameters were estimated to minimize the error. Animals were observed for any behavioural changes and mortality up to 72hrs after experiment.[14] No mortality was observed in the animals even after observation for a period of 72hrs. Hence the doses used in this study were not toxic.

Experimental animal groups

Thirty-six animals were divided in 9 groups (All the standard and test drugs were dissolved in distilled water). Two standard drugs were used in this study to compare the efficacy of test drug and to find out the possible mechanism of action and also most of the studies have chosen furosemide and hydrochlorothiazide as standard drugs. Doses of furosemide (20mg/kg) and hydrochlorothiazide (10mg/kg) were selected based on previous studies.[15,16] Test doses 150, 300 and 450mg/kg of leaf extracts were selected based on previous studies.[17,18]

Total 9 groups

1. Distilled water (DW), orally, once daily for 7 days (control)
2. Furosemide 20mg/kg orally, once daily for 7 days (standard)
3. Hydrochlorothiazide 10mg/kg orally, once daily for 7 days (standard)

Aqueous leaf extract of Sesbania grandiflora Linn.

4. 150mg/kg(AE-150), orally, once daily for 7 days
5. 300mg/kg(AE-300), orally, once daily for 7 days
6. 450mg/kg (AE-450), orally, once daily for 7 days

Methanolic leaf extract of Sesbania grandiflora Linn.

7. 150mg/kg(ME-150), orally, once daily for 7 days
8. 300mg/kg (ME-300), orally, once daily for 7 days
9. 450mg/kg (ME-450), orally, once daily for 7 days

Following indices were calculated from above parameters:

1. *Natriuretic activity:* The ratio Na^+/K^+ is calculated for natriuretic activity. Values greater than 2.0 indicate a favorable natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect.[11]
2. *Saluretic activity:* It is the sum of Na^+ and Cl^-
3. *Carbonic anhydrase inhibition:* It is calculated by $Cl^- / (Na^+ + K^+)$. 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.
4. *Diuretic index or diuretic action:* It is the ratio between urine volume of test group and control group.
5. *Saluretic index:* It is the ratio between saluretic activity of test group and control group.[19]
6. *Natriuretic index:* It is the ratio between natriuretic activity of test group and control group.

Statistical analysis

All the statistical tests were done by using SPSS version 6. The level of significance was 5% ($p \leq 0.05$). Results were analysed between the groups by One Way Analysis of Variance (ANOVA). Results were analysed within the group from day 1 to day 7 by Paired 't' test.

RESULTS

Saluretic activity= $(Na^+ + Cl^-)$; Natriuretic activity= (Na^+/Cl^-); Diuretic action or index = Urine volume of test group/Urine volume of control group; Carbonic anhydrase inhibition= $Cl^- / (Na^+ + K^+)$; Saluretic index= Saluretic activity of test group/ Saluretic activity of control group; Natriuretic index= Natriuretic activity of test group / Natriuretic activity of control group.

Table 1: Effect of *Sesbania grandiflora* linn leaf extracts on diuresisin wistar rats.

Groupsmg/kg	Saluretic activity	Natriuretic activity	Diuretic action or index	Carbonic anhydrase inhibition	Saluretic index	Natriuretic index
DW	200.03	1.69	1	0.82	1	1
Furo-20	356.18	2.21	3.18	0.80	1.78	1.31
HCT-10	299.71	2.10	2.48	0.80	1.50	1.24
AE-150	211.06	1.79	1.09	0.81	1.06	1.06
AE-300	252.66	1.97	1.85	0.80	1.26	1.16
AE-450	294.22	2.10	2.41	0.81	1.47	1.25
ME-150	221.55	1.80	1.34	0.81	1.11	1.06
ME-300	272.56	2.01	2.13	0.80	1.36	1.19
ME-450	327.98	2.19	2.80	0.80	1.64	1.30

DW = Distilled Water; Furo-20 = Furosemide 20mg/kg; HCT-10 = Hydrochlorothiazide 10mg/kg; AE-150= Aqueous leaf extract of *Sesbania grandiflora Linn* 150mg/kg; AE-300= Aqueous leaf extract of *Sesbania grandiflora Linn* 300mg/kg; AE-450= Aqueous leaf extract of *Sesbania grandiflora Linn* 450mg/kg; ME-150= Methanolic leaf extract of *Sesbania grandiflora Linn* 150mg/kg; ME-300= Methanolic leaf extract of *Sesbania grandiflora Linn* 300mg/kg; ME-450= Methanolic leaf extract of *Sesbania grandiflora Linn* 450mg/kg.

Effect of drugs on body weight

The day 1 and day 7 showed statistically significant increase in mean body weight ($p<0.05$) from basal to post treatment in distilled water treated group. No change in body weight in group treated with AE-150 ($p>0.05$). But, rest all groups showed statistically significant decrease in mean body weight ($p<0.05$). Highest decrease in body weight was observed with furo-20 treated group (2%) and lowest decrease was with group treated with AE-150 (0.1%), whereas increase (0.7%) in body weight was observed in group treated with DW.

Effect of drugs on urine volume

The urine volume differs between day 1 and day 7, there was no significant difference observed ($p>0.05$) between day1 and day7 at each dose in all groups. It suggests the effect of drugs on urine volume was consistent from day 1 to day 7. Order of effect of aqueous extract on urine volume (day 1 and 7): AE150 <AE-300 <AE450, Order of effect of methanolic extract on urine volume (day 1 ans 7): ME-150 <ME-300 <ME450.

Effect of drugs on sodium excretion

When compared to negative control all the drugs produced significant sodium excretion ($p<0.05$). Maximum sodium excretion obtained from group treated with standard drug furo-20 (164.65mEq/lit), next highest with the ME-450 (151.74) then least with AE-150 and DW on 7th day. Order of effect aqueous extract on sodium excretion (day 1 and 7): AE-150 <AE-300 <AE450. Order of effect methanolic extract on sodium excretion (day 1 and 7): ME-150 <ME-300 <ME450. No significant difference was observed in sodium excretion ($p>0.05$) between day 1 and day 7 at each dose in all groups in Paired 't' test. It suggests the effect of drugs on

sodium excretion was consistent throughout the study period.

Effect of drugs on potassium excretion

Like urine volume and sodium excretion, methanolic extract has showed significant potassium excretion than aqueous extract. In methanolic extract potassium excretion has started at the dose of 150 mg/kg (54.67mEq/lit). Then dose dependant increase in potassium excretion was observed. Order of effect aqueous extract on potassium excretion (day 1 and 7): AE150 <AE-300 <AE450. Order of effect methanolic extract on potassium excretion (day 1 and 7): ME-150 <ME300 <ME450.No significant difference observed in potassium excretion ($p>0.05$) between day 1 and day 7 at each dose in all groups in Paired 't' test. It suggests the effect of drugs on potassium excretion was consistent.

Effect of drugs on chloride excretion

Maximum chloride excretion obtained from the group treated with standard drug furo-20 (191.08mEq/lit). Another standard drug HCT-10 produced 163.06mEq/lit chloride excretion. Order of effect aqueous extract on chloride excretion (day 1 and 7): AE150 <AE-300 <AE450. Order of effect methanolic extract on chloride excretion (day 1 and 7): ME-150 <ME300 <ME450. No significant difference observed in chloride excretion ($p>0.05$) between day1 and day 7 at each dose in all groups. It suggests the effect of drugs on chloride excretion was consistent.

Effect of drugs on natriuretic activity

The ratio Na^+/K^+ is calculated for natriuretic activity. Results suggested that Furo-20, ME-450, HCT-10, AE-450, ME-300 have favourable natriuretic activity with values greater than 2.0. AE-300 natriuretic activity almost equal to 2. No group produced more than 10.0, hence there is no potassium sparing effect for this plant extract. Order of natriuretic activity among groups: Furo-20 (2.21) >ME-450 (2.19) >HCT-10 (2.10) = AE-450 (2.10) >ME-300 (2.01) >AE-300 (1.97) >ME-150 (1.80) >AE-150 (1.79) >DW (1.69).

Effect of drugs on diuretic index or diuretic action

It is the ratio between urine volume of test group and control group. Highest diuretic index was observed with furosemide group that at 3.18. Among test drugs ME-450 showed highest diuretic index (2.80). Order of diuretic index among groups: Furo-20 (3.18) >ME-450 (2.80) >HCT-10 (2.48) >AE-450 (2.41) >ME-300 (2.13) >AE-300 (1.85) >ME-150 (1.34) >AE-150 (1.09) >DW (1).

Effect of drugs on Carbonic anhydrase inhibition

Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. Carbonic anhydrase activity starts at ratio less than 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition is present. In this study all the values were between 1.0 and 0.8. Hence these drugs don't have action on carbonic anhydrase inhibition.

Effect of drugs on saluretic activity

It is the sum of Na^+ and Cl^- excretion. Highest saluretic activity observed with furosemide (356.18 mEq/lit). Among test drugs highest saluretic activity was observed with ME-450 (327.98mEq/lit). Order of saluretic activity among groups: Furo-20 (356.18mEq/lit) >ME-450 (327.98mEq/lit) >HCT-10 (299.71mEq/lit) >AE-450 (294.22mEq/lit) >ME-300 (272.56mEq/lit) >AE-300 (252.6mEq/lit) >ME-150 (221.55mEq/lit) >AE-150 (211.06mEq/lit) > DW (200.03mEq/lit).

Effect of drugs on saluretic index

The Highest saluretic index was observed with furosemide (1.78). Among test drugs highest saluretic index was observed with ME-450 (1.64). Order of saluretic index among groups: Furo-20 (1.78) >ME-450 (1.64) >HCT-10 (1.50) >AE-450 (1.47) >ME-300 (1.36) >AE-300 (1.26) >ME-150 (1.11) >AE-150 (1.06) >DW (1).

Effect of drugs on natriuretic index

The Highest natriuretic index was observed with furosemide (1.31). Among test drugs highest natriuretic index was observed with ME-450 (1.30). Order of saluretic activity among groups: Furo-20 (1.31) >ME-450 (1.30) >HCT-10 (1.24) = AE-450 (1.24) >ME-300 (1.19) >AE-300 (1.16) >ME-150 (1.06) = AE-150 (1.06) >DW (1).

DISCUSSION

This study was carried out in wistar albino rats, comparing the diuretic activity of aqueous and methanolic leaf extracts of *Sesbania grandiflora Linn* with standard drugs furosemide and hydrochlorothiazide using metabolic cage.

Furosemide is an inhibitor of Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the loop of henle.[20] In addition to its prominent tubular action, it causes acute changes in renal and systemic haemodynamics due to increased local prostaglandin synthesis. Intravenous administration causes prompt increase in systemic venous capacitance and decreases left ventricular filling pressure. This causes quick relief of left ventricular failure and pulmonary edema. Hydrochlorothiazide is an inhibitor of Na^+-Cl^- cotransporter proximal to the sodium and potassium exchange region in the distal tubule.[21] It exerts only a moderate diuretic effect. It is commonly used as an anti-hypertensive.

Diuretics are the mainstay of treatment in many conditions like hypertension, heart failure, nephritic syndrome, cirrhosis of liver, renal failure, eclampsia etc.[6] A number of diuretics like mannitol, thiazides and furosemide are used in practice. Still there is a need for more effective and less toxic diuretics. *Sesbania grandiflora Linn* has chosen as a test drug because it is easily available and minimal pharmacological or clinical study was carried out to test the diuretic activity.

The earlier studies was reported methanolic extract in increasing doses there was decrease in urine volume and aqueous extract showed increase in urine volume as the dose increased.[17] In contrast, in the present study, there was increase in urine volume with increasing doses both with aqueous and methanolic extracts and also showed significant increase in urine volume with higher dose of methanolic extract (400mg/kg), but not with the lower dose (200mg/kg).[22] But in this study significant difference was observed with lower dose (150mg/kg) and increase in urine volume with increase in dose by methanolic leaf extract of doses 30, 60, 90 and 120 mg/kg produced significant urine volume compared to negative control but not attained urine volume as that of furosemide.[18] In this study, the methanolic extract in a dose of 150, 300 and 450 mg/kg were used, the significant urine volume was observed with lower dose, and increased with increasing doses.

In this study the sodium excretion with methanolic extract was high compared with the aqueous extract similar to that of earlier study[17] but in contrast to this study, sodium excretion was significantly decreased with higher doses of methanolic extract. Dose dependant sodium excretion of aqueous extract in this study was supported previous studies.[17] The sodium excretion was relatively less compared to this study where there was significant sodium excretion. It may be due to use of higher dose of methanolic extract in this study.[18]

The potassium excretion in the urine was statistically not significant in all the groups of methanolic and aqueous extracts of Sesbania grandiflora Linn. in Rats.[17] Where lower dose of methanolic extract (150mg/kg) showed better response than higher dose (300mg/kg) but in aqueous extract treated groups, response was increased with increasing doses. But in this study there was significant increase in potassium excretion as the dose increases both in aqueous and methanolic extract treated groups. The cause in the variation of results may be due variation in the concentration of phytoconstituents and part of the plant used.

The phytochemical constituents of *Sesbania grandiflora Linn* are tanins, flavonoids, coumarins, steroids, triterpens and alkaloids. The alkaloids are generally found in traces. The saponins are present more in methanolic extracts than aqueous extract.[23,24] The study suggests saponins, flavonoids, steroids, triterpens, tannins and alkaloids have diuretic activity.[25,26]

The presence of saponin might be responsible for salurtic activity by modulating renal sodium excretion. Presence of phenolic compounds, organic acids and polar compounds such as flavonoids and and steroidal saponins are responsible for diuretic activity.[19]

These substances might be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically. The best diuretic effects could be associated to the flavonoid content.[27] The diuretic effect may be produced by stimulation of regional blood flow or initial vasodilatation, or by causing inhibition of tubular reabsorption of water and electrolytes, the result in both cases being dieresis.[28]

Some herbal diuretics induce diuresis by stimulating the thirst center in the hypothalamus and thereby enhancing the fluid intake. This mode of action is unlikely to be operative in the present study since the rats had no access to fluid intake during the experimental period (24Hrs).[29] All the extracts were not accompanied with reduction in urinary K^+ level. These data indicate that they are not acting as potassium sparing diuretics.[30] Moreover no drug produced natriuretic activity value more than 10 and test drug excreted potassium, dose dependant manner (no sparing of potassium) hence it excludes the potassium sparing effect.

Carbonic anhydrase inhibition values were observed between 1.0 and 0.8. Hence it excludes the action of carbonic anhydrase inhibition. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

CONCLUSION

All the electrolytes concentration was increased in the urine, hence excluding osmotic diuretic action. Diuretic index, saluretic activity, saluretic index and natriuretic index of ME-450 were more than the value of hydrochlorothiazide hence excludes thiazide diuretic action. But these values were less than furosemide. But further study with higher doses of the test extract may produce effects similar to that of furosemide. For this further study are needed to be carried out with higher doses greater than 450 mg/kg. So the data here supports that the test drug may have action like loop diuretic (furoseimde). For understanding the mechanism of action more trials are needed. Further studies will be necessary to substantiate the potential diuretic value of this plant and its phytoconstituents and to evaluate the effects of long-term administration.

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