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Diuretic activity of ethanolic extract of pods of *Acacia concinna* Linn. in Wistar albino rats

Vanishree Subhanreddy, Dr. Arati Malpani and Irshad Khan

Abstract
Introduction: Diuretics is any substance that promote diuresis, the increased production of urine. Diuresis is an important process for excreting catabolites, maintaining the hydro electrolyte equilibrium and eliminating toxic substances. Diuretics are prescribed in various conditions like congestive heart failure, cirrhosis of liver, nephrotic syndrome, hypertension, hyperkalemia, to treat ingestion of toxic anions etc.
Methods: EEAC was evaluated for phytochemical analysis, toxicity studies and diuretic activity (Lipschitz value). The diuretic effect of the EEAC was evaluated by various parameters such as urine volume, urinary electrolytes (Na^+, K^+, Cl^+), saluretic effect, natriuretic effect, and CAI (carbonic anhydrase inhibitory) in Wister albino rats. Different concentrations of EEAC (500 & 1000 mg/kg) and Furosemide (20 mg/kg) were administered orally to Wister albino rats and their urine output was monitored at several intervals of time (5 h and 24 h).
Results: Results of acute oral toxicity studies showed that, the extract was safe up to a dose level of 5000 mg/kg weight. Phytochemical analysis shows presence of flavonoids, alkaloids, saponins, tannins and phenolic compounds. The highest dose of EEAC significantly ($p<0.05$) and markedly increased the urine output. Urinary electrolyte excretion was significantly ($*p<0.05$) increased at 1000 mg/kg when compared to the control. EEAC also showed a significant ($**p<0.01$) saluretic and good natriuretic effect at 1000 mg/kg, which may be due to flavonoids, saponins and alkaloids present in the extract as a phytoconstituents. This study supports the plant's traditional use as a diuretic.
Conclusion: The ethanolic extract of pods of *Acacia concinna* showed significant diuretic activity, good diuretic action, good saluretic effect, and good natriuretic action which may be because of phytoconstituent (Flavonoids, saponins, alkaloids) present in the extract. The plant's historical use as a diuretic is supported by this study. Further study is required to fractionate and isolate the molecules from extract, as well as to determine the exact mechanism of action of the molecule for diuretic activity of the molecule for diuretic activity.

Keywords: *Acacia concinna* L., diuretic activity, furosemide, saluretic, natriuretic, carbonic anhydrase

Introduction
Diuretics is any substance that promote diuresis, the increased production of urine. Diuresis is an important process for excreting catabolites, maintaining the hydro electrolyte equilibrium and eliminating toxic substances [1].
Diuretics are prescribed in various conditions like congestive heart failure, cirrhosis of liver, nephrotic syndrome, hypertension, hyperkalemia, to treat ingestion of toxic anions etc. The adverse effect associated with diuretics are hyperyruricemia, hypercalciuria, hypomagnesaemia, hypokalemia, ototoxicity, hyperglycemia, erectile dysfunction, hypersensitivity reactions etc. [2]. Hence, the search for better drugs with lesser adverse effects, which are more efficacious and economical is need of the hour. Plants are source for various drugs. They offer an untapped potential for newer drugs [2]. Nowadays, 99% of the world is getting attention for natural remedies. Natural herbs are helpful in the development of advance medicines and treatments in future. The therapeutic effects of these natural remedies may be less pronounced at times than synthetic drugs, but the probability of adverse symptoms were minimum. Medicinal herbs are the most significant source of diuretics. Many researchers demonstrated the studies of herbal plants used in traditional medicines which are used as diuretics have showed progressive elevation in the last decades and might be a precious tool used in human pathology treatment [3].
There exist a large number of studies that support the diuretic effects of traditional herbal medicines. So there is a need to develop diuretic molecules from plants or herbs [4].

Acacia concinna Linn. (Leguminosae) is a medicinal plant that grows in tropical rainforests of southern Asia and the fruits of this plant are used for washing hair, for promoting hair growth, expectorant, emetic, and purgative. The pods of this plant contain several saponins based on acacic acid, previous chemical examination resulted in the identification of flavonoids 1 and monoterpenoids [5].

There are versatile uses of this plant (Leaf, stem and pods) in different aspects of life. The leaves and pods are used to treat cuts, wounds, oral diseases and antidandruff. Leaves of shikakai act as a purgative, liver stimulant and improves taste. The decoction of shikakai pods used to treat constipation, abdominal pain, indigestion and flatulence and infusion of the leaves of the plant has also been used for therapy of jaundice in the traditional Indian medicines. The pulp of the fruit, without the seeds, is used as a diuretic and emetic, while the seeds are reputed to make delivery [6, 7].

The use of traditional medicines as a diuretic agent has been increasing in present years. In Indian traditional medicine, the plant *Acacia concinna* L. is claimed to possess powerful diuretic activity. However, the diuretic potential of this plant has not yet been investigated. Therefore, the present study was aimed to evaluating the diuretic activity of pods of *Acacia concinna* L. in wistar albino rats.

Materials and Methods

Preparation of plant extract

The fruit pods of *Acacia concinna* L. will be collected, shade dried. The dried pods will be pulverized separately into coarse powder by a mechanical grinder. The resulting *Acacia concinna* pods powder (100 g) was defatted with 150 ml of petroleum ether and extracted with ethanol by hot percolation method using a Soxhlet apparatus at 40 °C to obtain the ethanolic extract of the plant. The filtrate of the extract was concentrated and dried at a temperature of 30 °C. The percentage yield was calculated and reported.

Animals

Wister albino rats (150-250 g) of either sex were obtained from Mahaveer Enterprises, Hyderabad, Telangana, India (1656/PO/Bt/S/12/CPCSEA). They were maintained in an HKES MTRIPS animal house at a temperature of 25±1 °C and a relative humidity of 45% to 55% under a 12-hours light and 12-hour dark cycle. The animals had a free access to food pellets, and water was available *ad libitum.* Prior permission from the IAEC was obtained for the conduct of the experiments (IAEC approval no. HKES/MTRIPS/IAEC/122/2021-22).

Acute oral toxicity test

Acute oral toxicity studies were conducted as per OECD (Organization for Economic Cooperation and Development) guideline 425 (8). Healthy rats (Wister Albino rats), 150 to 200 gm (8 to 12 weeks old), were used for the study. The food, but not the water, was withheld for overnight. Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animals is determined and the dose is calculated according to body weight. Initiating at a dose 175 mg/kg, p.o. When no abnormality or death was observed, the next doses of 550, 1750, and 5000 mg/kg were chosen. At the dose of 5000 mg/kg, an additional four rats were dosed. All the animals were observed for initially 30 m and then 24 h for behavioral, neurological, and autonomic profiles and for lethality or death over the next 48 h.

Preliminary phytochemical screening

The pods of EEAC qualitatively tested for the detection of alkaloids, saponins, flavonoids, tannins, and phenolic compounds [9].

Diuretic activity

Wistar albino rats weighing 150-250 g were selected, divided into four groups six rats in each group, control group, Furosemide-treated groups, and EEAC-treated groups (500 & 1000 mg/kg). The control group was treated 10 ml/kg of body weight of normal saline; the standard drug-treated groups was treated with Furosemide 20 mg/kg orally. The EEAC-treated groups were treated with 500 and 1000 mg/kg doses of ethanolic extract of *Acacia concinna* (EEAC). All drugs were given orally. Each animal was placed in isolation in metabolic cages and fasted overnight with free access to water. Urine samples were collected after 5 h and 24 h of the last dose. The urine samples were filtered and finally stored at 20°C for electrolyte analysis [10].

Measurement of urine parameters

Total urine volume was measured after 5 h and 24 h for all the rats. The total concentrations of electrolytes (sodium, potassium, chloride ions) in urine samples (over 24 h) were determined by the Ion Selective Electrode method described in the instruction manual of the biochemical kits.

Diuretic action and activity (Lipschitz value)

The diuretic action and diuretic activity (Lipschitz value) were calculated from fallowing formula

$$\text{Diuretic action} = \frac{\text{Urine volume of test group}}{\text{Urine volume of control group}}$$

$$\text{Diuretic Activity} = \frac{\text{Urine output in test group}}{\text{Urine output in standard group}}$$

from the ratio of urine volume in the test group and the control and furosemide groups, respectively. Before the experiment began, it was decided the diuretic activity would be regarded as “moderate” and “good” if the values were 0.72-1.00 and 1.00-1.5 [11].

Saluretic, natriuretic and carbonic anhydrase inhibition

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saluretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity (Values greater than 2.0 indicate a favorable natriuretic effect). The ratio $cl^-/(Na^+ + K^+)$ was calculated to estimate carbonic anhydrase inhibition (Carbonic anhydrase inhibition can be excluded at ratios between 1.0 to 0.8 with decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed) [11].

Statistical analysis

Data were expressed as Mean ± SEM and statistical analysis was carried out by one-way Analysis of Variance (ANOVA) followed by Dunnett’s test.

Results

Acute toxicity test

In acute oral toxicity studies, no behavioral or autonomic abnormalities, as well as no mortality, were observed in any of the rats treated with the ethanolic extract of pods of *Acacia concinna* up to the level of 5000 mg/kg. The extract was

found to be safe up to the maximum dose level of 5000 mg/kg of body weight.

Preliminary phytochemical screening

The percentage yield of pods of EEAC was 25%. The preliminary phytochemical assessment of EEAC showed the presence of alkaloids, saponins, flavonoids and tannins, phytosterols, phenolic compounds (Table 1).

Effect on urine volume, diuretic action and diuretic activity (Lipschitz value)

The details of urine volume, diuretic action, and diuretic activity were presented in Table 2. The results were revealed that *Acacia concinna* exhibited dose-dependent diuretic activity at 5 h and 24 h. EEAC at both the dose (500 and 1000 mg/kg) and furosemide (20 mg/kg) significantly increased the urine output (*p<0.05, **p<0.01 and ***p<0.001, respectively) at 5 h and 24 h when compared to control rats. On the basis of urine volume in rats, the diuretic action of the EEAC at 500 and 1000 mg/kg was 1.27 and 1.99 at 5 h and 1.32, 1.71 at 24 h respectively. The diuretic activity (Lipschitz value) of *Acacia concinna* exhibits moderate diuretic activity, the ratios were 0.58 and 0.90.

Effects on urinary electrolyte excretion

EEAC at both the doses (500 and 1000 mg/kg) increased the urinary excretion of Na^+ and Cl^- compared to control; the extract response was dose dependent. The EEAC at a dose of 1000 mg/kg significantly (*p<0.05) increased the urine excretion of Na^+ and Cl^- (Table 3).

Effects on saluretic, natriuretic and carbonic anhydrase inhibition

Both the 500 mg/kg and 1000 mg/kg dosages of the EEAC demonstrated saluretic activity, only the 1000 mg/kg dose significantly (***p0.001) increased saluretic activity in compared to the control group (Table 4). EEAC at 1000 mg/kg exhibited favorable natriuretic response which was indicated by a ratio > 2.0. The CAI ratios of 500 and 1000 mg/kg were 0.90 and 0.76, respectively (Table 4), EEAC did not exhibit any carbonic anhydrase inhibition.

Disscussion

Diuretics are medications that increase urine flow and salt excretion, and they are used to control body fluid content in a variety of clinical situations like hypertension, nephrotic syndrome, heart failure, and cirrhosis of liver [12].

In the present study, the diuretic effects of both doses of EEAC were evaluated in Wistar albino rats. The results indicated that EEAC at both doses (500 and 1000 mg/kg) significantly (*p< 0.05 and **p< 0.01, respectively) increased urine volume in a dose-dependent manner over a period of 5 h and 24 h (Table 2). EEAC at 500 and 1000 mg/kg compared with standard furosemide, showed "moderate" diuretic activity (Lipschitz value) at 5 h and 24 h, and also showed good diuretic action when compared with control (Table 2). EEAC showed an increase in the urinary excretion of Na^+, K^+, and Cl^- in a dose-dependent manner when compared to control (Table 3).

EEAC at a dose of 1000 mg/kg showed a significant (***p< 0.001) saluretic effect when compared to the control (Table 4). Natriuretic activity was determined by calculating the ratio Na^+ /K^+ and a value greater than 2.0 indicated a favourable natriuretic effect [13]. Na^+ /K^+, indicates that more Na^+ is excreted than K^+, which is seen as a highly positive profile for diuretics. In the present study, EEAC at 1000 mg/kg showed a value greater than 2.0, which indicates a good natriuretic effect (Table 4). The diuretic effect was found to be more significant at a higher dose (1000 mg/kg) compared to the control, which might be due to the increased concentration of active component present in the extract. The ratio of Cl^-/(Na^++K^+) is calculated for CAI, and CAI can be excluded at ratios between 1.0 and 0.8; with decreasing ratios, slight to strong CAI can be assumed [13]. In the present study, EEAC at 500 and 1000 mg/kg doses did not exhibit any CAI, as the values of the CAI ratio were 0.90 and 0.76, respectively (Table 4).

In the present study, furosemide was used as a standard drug. Furosemide increases urinary excretion of sodium by inhibiting the $Na^+/K^+/Cl^-$ co-transporter system in the thick ascending limb of loop of the Henley. The $Na^+/K^+/Cl^-$ co-transporter play a vital role in salt reabsorption into the interstitium blood. When salt reabsorbing into the interstitium blood the electrolyte concentration in blood increases. Furosemide block the function of $Na^+/K^+/Cl^-$ co-transporter and there by the salt reabsorption in to the interstitium blood. Which leads to automatic increase in urinary electrolyte concentration [14]. Our study results revealed EEAC also increased urine volume and urinary Na^+, K^+ and Cl^-, so may acts same as that of the furosemide.

Table 1: Phytochemicals constituents present in ethanolic extract of pods *of Acacia concinna L.*

Test	Ethanolic extract
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Phenolic compounds	+
Terpenoids	-
Phytosterols	+

(+) - Positive, (–) – Negative

Table 2: Effect of ethanolic extract of Pods of *Acacia concinna* on urine volume Diuretic action & diuretic activity at 5 h and 24 h

Groups	At 5 h after drug administration			At 24 h after drug administration		
	Urine volume (mL)	Diuretic action[a]	Diuretic activity[b]	Urine volume (mL)	Diuretic action[a]	Diuretic activity[b]
Control (10 ml/kg)	1.18±0.21	1.00	——	4.15±0.42	1.00	——
Furosemide (20 mg/kg)	2.60±0.20***	2.20	1.00	8.05±0.24***	1.93	1.00
EEAC (500 mg/kg)	1.51±0.20*	1.27	0.58	5.48±0.55*	1.32	0.68
EEAC (1000 mg/kg)	2.35±0.22**	1.99	0.90	7.10±0.43**	1.71	0.88

n=6; Values are expressed as mean ± S.E.M; *p < 0.05, **p < 0.01, ***p < 0.001 compared to control group.
EEAC – Ethanolic extract of *Acacia concinna* Linn.
a Diuretic action = urine volume of test group/urine volume of control group.
b Diuretic activity = urine volume of test group/urine volume of furosemide group.

Table 3: Effect of ethanolic extract of pods of *Acacia concinna* on urinary electrolyte (Na+, K+ and Cl-) at 24 h of urine sample

Groups	Urinary Na^+ (mmol/L)[a]	Urinary K^+ (mmol/L)[a]	Urinary Cl^-(mmol/L)[a]	Na^+ index[b]	K^+ index[b]	Cl^- index[b]
Control (10 mg/kg)	82.62±6.77	53.53±2.96	102.4±11.04	1.00	1.00	1.00
Furosemide (20 mg/kg)	178.9±7.15****	79.83±7.32*	189.1±13.12**	2.16	1.49	1.84
EEAC (500 mg/kg)	112.3±14.56	65.27±4.05	163.0±14.04*	1.35	1.21	1.59
EEAC (1000 mg/kg)	160.1±14.93***	72.90±7.28	177.7±19.54**	1.93	1.36	1.73

a n=6; Values are expressed as mean ± S.E.M; *$p < 0.05$, **$p < 0.01$ compared to control group.
b Index = excretion in test group/excretion in control group.

Table 4: Effect of ethanolic extract of pods of *Acacia concinna* L on saluretic, natriuretic and carbonic anhydrase inhibition activity at 24 h of urine sample

Groups	Saluretic effect (Na + Cl)[a]	Natriuretic effect (Na/K)[a]	CAI [Cl/(Na + K)][a]	Saluretic index[b]	Natriuretic index[b]	CAI index[b]
Control (10 ml/kg)	185.0±16.84	1.54±0.10	0.74±0.60	1.00	1.00	1.00
Furosemide (20 mg/kg)	368.0±19.51****	2.31±0.17*	0.73±0.03	1.98	1.50	0.98
EEAC (500 mg/kg)	275.2±26.62*	1.70±0.17	0.90±0.06	1.48	1.10	1.21
EEAC (1000 mg/kg)	337.8±30.31***	2.45±0.30*	0.76±0.05	1.82	1.59	1.02

a n=6; Values are expressed as mean ± S.E.M; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control group.
b Index = excretion in test group/excretion in control group.
CAI – Carbonic anhydrase inhibition

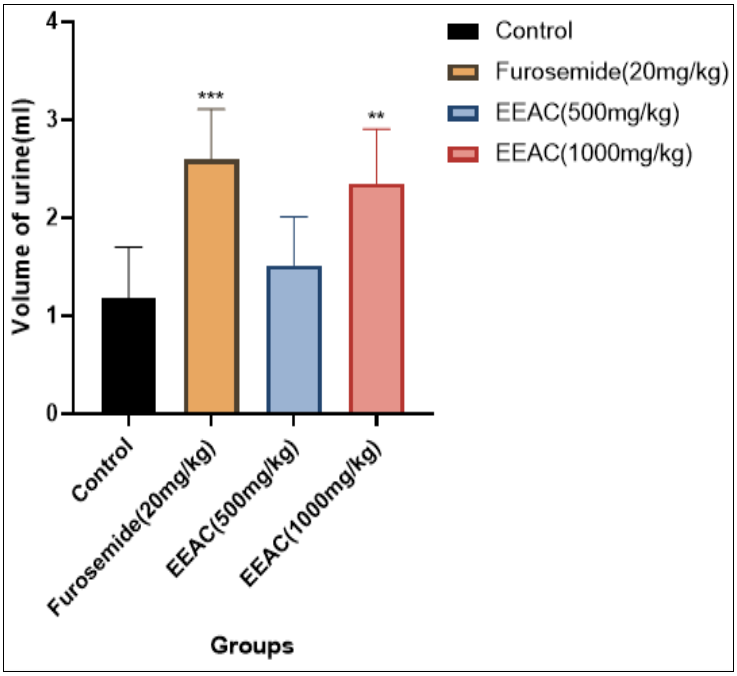

Fig 1: Effect of EEAC on urine volume at 5h

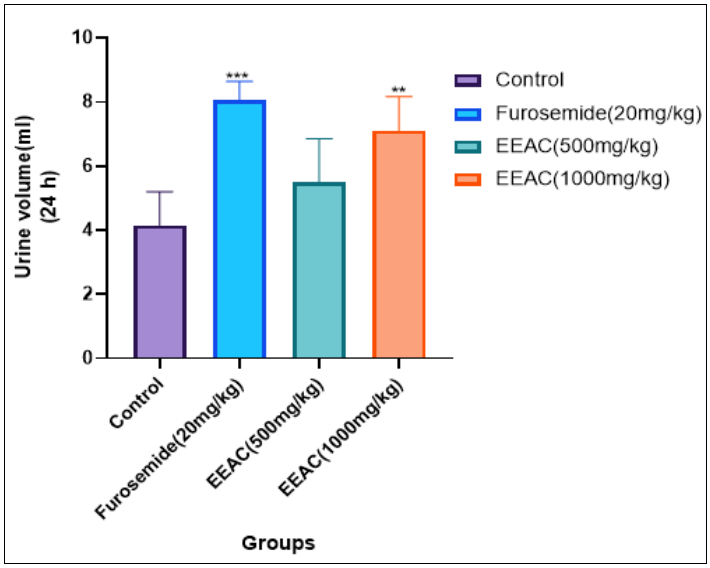

Fig 2: Effect of EEAC on urine volume 24h

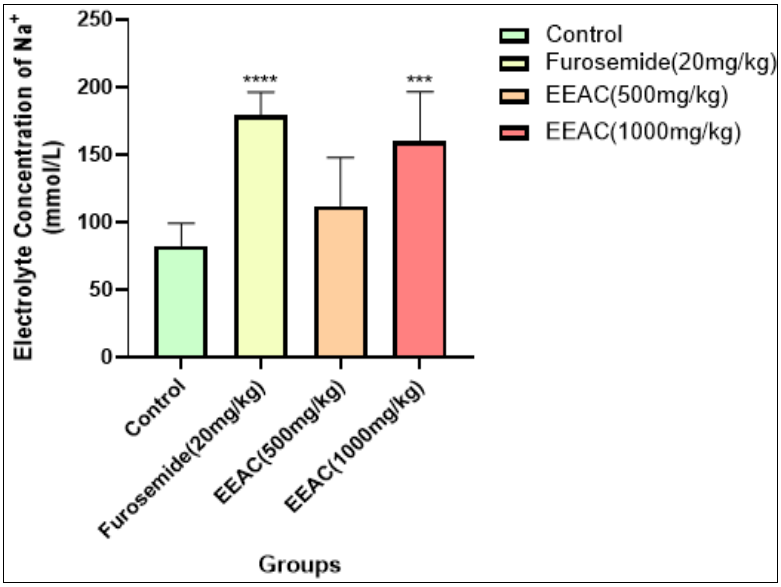

Fig 3: Effect of EEAC on urinary sodium

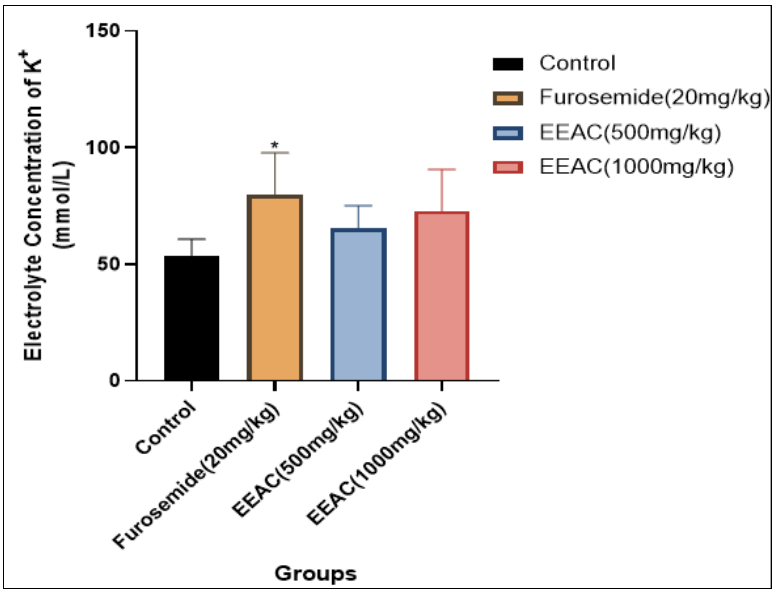

Fig 4: Effect of EEAC on urinary potassium

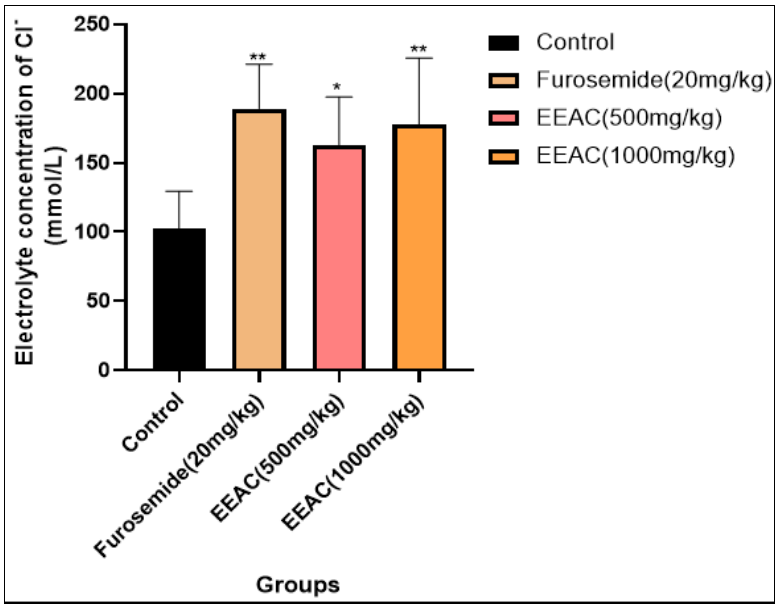

Fig 5: Effect of EEAC on urinary chloride

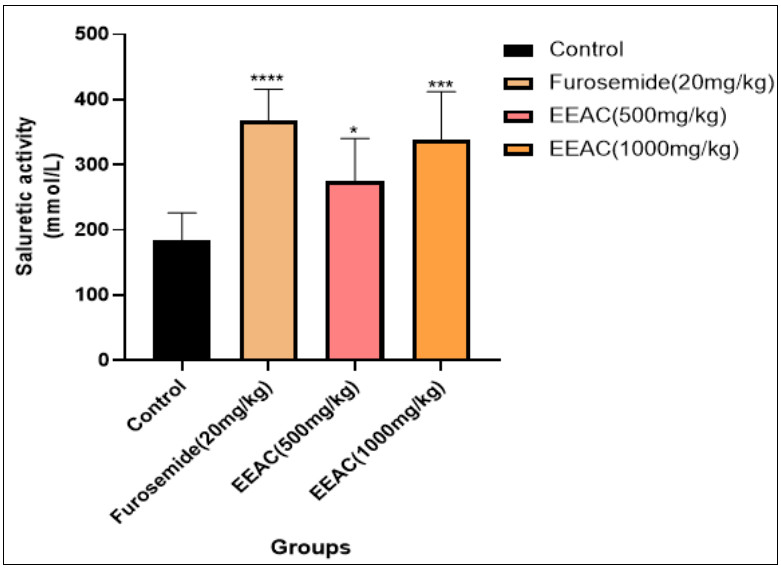

Fig 6: Effect of EEAC on saluretic activity

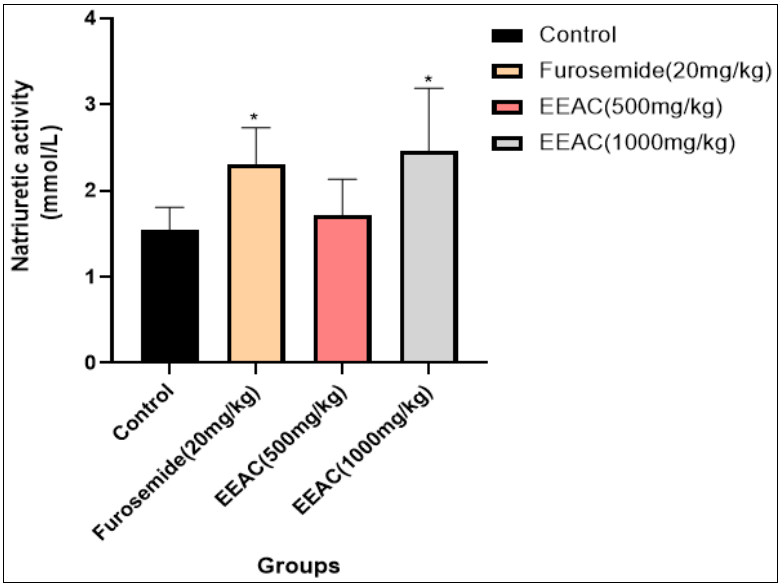

Fig 7: Effect of EEAC on natriuretic activity

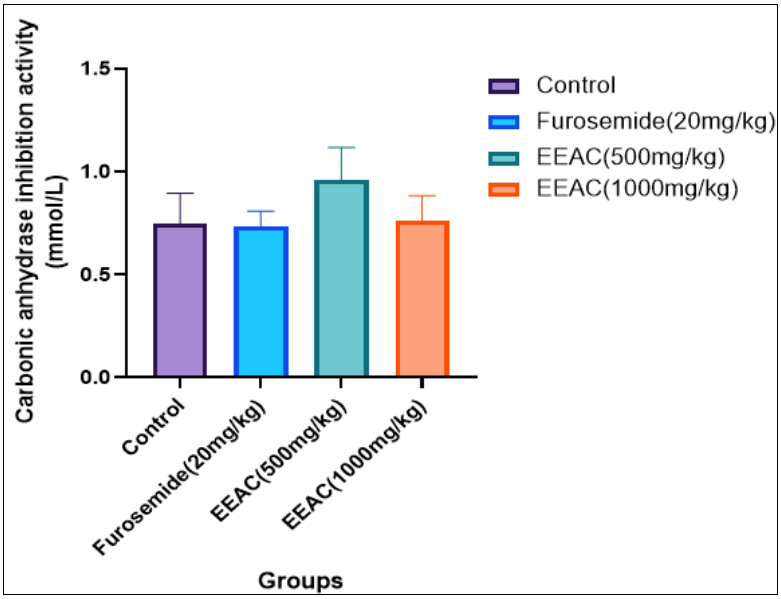

Fig 8: Effect of EEAC on carbonic anhydrase inhibition

Flavonoids affect several renal factors that promote diuresis and natriuresis, which may contribute to their well-known hypertensive effect. Flavonoids prevent or attenuate the renal injury associated with arterial hypertension, both by

decreasing blood pressure and by acting directly on the renal parenchyma. Flavonoids also improve cisplatin- or methotrexate-induced renal damage. Some studies have proved that glomerular filtration rate significantly increased after administration of flavonoids which lead to diuretic actions [15]. EEAC shows presence of flavonoids as phytoconstituent which might be responsible for its diuretic activity.

Conclusion

The ethanolic extract of pods of *Acacia concinna* showed significant diuretic activity, good diuretic action, good saluretic effect, and good natriuretic action which may be because of phytoconstituent (flavonoids) present in the extract. The plant's historical use as a diuretic is supported by this study. Further study is required to fractionate and isolate the molecules from extract, as well as to determine the exact mechanism of action of the molecule for diuretic activity. of the molecule for diuretic activity.

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Declaration of interest

Authors declare no conflict of interest.

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