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Diuretic activity and acute oral toxicity study of ethanolic extract of leaves of *Ficus benjamina* L. in Wistar albino rats

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Abstract
The main goal of the study was to know the diuretic activity of the ethanolic extract of leaves of *Ficus benjamina L.* The diuretic activity of ethanolic extract of EEFB was tested at the doses of 200 and 400 mg/kg. The diuretic activity was evaluated by various parameters such as volume of urine output, diuretic action, diuretic activity (Lipschitz value), concentration of urinary electrolytes (sodium, potassium and chloride), saluretic activity, natriuretic activity and carbonic anhydrase inhibition (CAI). The ethanolic extract of leaves of *Ficus benjamina L.* (EEFB) at 200 and 400 mg/kg showed a significant increase in urine output and also showed good diuretic action and diuretic activity (Lipschitz value) when compared with the control group in a dose-dependent manner. In concentration of urinary electrolytes the EEFB at the dose of 400 mg/kg showed a significant increase in Na^+, $K^{+,}$ and Cl^- and at 200 mg/kg showed significant activity only at urinary excretion of Cl^-. In saluretic and natriuretic activity, the EEFB showed good effect at 400 mg/kg and EEFB at 200 mg/kg showed little saluretic activity but didn't show natriuretic activity. EEFB did not exhibit any CAI activity.

Keywords: *Ficus benjamina L.* (EEFB), Diuretic activity, Saluretic activity, Natriuretic activity, Carbonic anhydrase inhibition

Introduction
Ficus benjamina L. belongs to the family Moraceae and is commonly known as weeping fig, is a multipurpose tree found in various parts of Pakistan. *Ficus benjamina L* is native to a large area including India, southern China, Southeast Asia, Malaysia, the Philippines, northern Australia, and the islands of the South Pacific [1]. *Ficus benjamina L.* is a very common houseplant found in temperate areas with elegant growth. It does best in sunny, bright conditions, but it also tolerates considerable shade. It requires an average amount of watering in summer. At night are favorable conditions for good appreciable growth in a short time. The plant is sensitive to cold and it should be protected from strong drafts. *Ficus benjamina L.* has been shown to effectively remove gaseous formaldehyde from indoor air [2]. The leaves are very sensitive to small changes in light. When it is turned or relocated, it reacts by dropping many leaves and replacing them with new leaves. The plant is sensitive to changes in environmental factors such as humidity and temperature. Shiny oval leaves can be categorized into the following classes depending on the species plain green, creamy yellow, marked with burgundy, green, yellow, or pink silver-white patterns. The *Ficus benjamina L.* plant is available as a natural-looking bush however;it is also grown on trunks that can be twisted, straight, or interwoven. Generally, branches droop slightly providing it a graceful green appearance. Leaves are oblong-ovate, leathery, 6-9 cm long, having a noticeable and somewhat slender point, rounded base. Petioles are 5-10 cm long while fruit is solitary, axillary, dark purple, stalkless, and fleshy when mature, rather spherical, and 1cm in diameter [3]. The roots and leaves boiles in oil form good applications for wounds and bruises. Leaves are applied to ulcers. Juice of the bark has a reputation for liver disease. *Ficus benjamina L.* is also used in rheumatic headache, and flatulent colic. When the baby's eyes get white, they mix some of the juice with the mother's milk and instill about two drops of this mixture into the eyes. It is also used as diuretic [4]. The plant has been reported to have hepatoprotective activity [5], metabolite profiling and inhibitory properties [6], phytochemical composition and antioxidant activity [7], allelopathic effect [8] on leaves extract of *Ficus benjamina L.* The plant has also been reported for chemical composition and biological studies [9], identification of alkaloids profile with higher antioxidant power [10], phytochemical and antibacterial studies of the fruit extract of *Ficus benjamina L.* [11]. Pharmacological potential and phytochemical profile [12], antioxidant, antibacterial, antiheamolytic [13].

Therefore the present study was conducted to evaluate the diuretic activity of *Ficus benjamina L.* for the authentication for its traditional use.

Materials and Methods

Collection of plant materials: *Ficus benjamina L*. plant leaves were collected from the local area of Kalaburagi, Karnataka, India. The plant was authenticated by the department of botany HKE'S Veeramma Gangasiri College of Women, Kalaburagi 585102, Karnataka.

Preparation of Extracts: *Ficus benjamina L*. leaves were washed with normal tap water and then again washed with distilled water and dried in the shade. The dried leaves were comminuted to fine powder. *Ficus benjamina L*. leaves powder (100gm) was defatted with 150ml of petroleum ether and extracted with ethanol by hot percolation method using Soxhlet apparatus at 40°C to obtain the ethanolic extract of the plant. The filtrate of the extract was concentrated and dried at under temperature of 30°C.

Acute oral toxicity studies: Acute oral toxicity was performed by using OECD guidelines-423 (Organization of Economic Co-Operation Development) - Fixed Dose Procedure. The purpose of this study is to allow the selection of the appropriate starting dose for the main study.
Acute oral toxicity of *Ficus benjamina Linn* was performed in Wistar Albino Rats. The rats were kept for 4 hr of fasting before the experiment and the body weight of the rats should be noted. Usually, rats weighing 180-250 gm were used for acute toxicity studies. The dose of the drug was given to each rat orally according to body weight. The test for acute toxicity was performed at 5, 50, 300, and 2000mg/kg oral doses of Ethanolic extract of *Ficus benjamina Linn* leaves. Food was given for1-2 hours after the administration of the drug.
During the first 4 hr. after the drug administration, animals were continuously observed for gross behavioral changes & and then observation was continued for 24 and 72 hr in regular intervals for 14 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, change in fur color, mortality, moribund stage, or death were observed [14].

Diuretic Activity: The method of Lipschitz [15] was employed for the assessment of diuretic activity. Wistar albino rats weighing 100-200g were selected. In this test, the twenty-four rats were randomly divided into four groups, containing six rats in each group.

- **Group I:** Control (Normal saline at a dose of 10 ml/kg, P.O.)
- **Group II:** Standard (Furosemide at a dose of 20 mg/kg, P.O.)
- **Group III:** EEFB (Low dose 200 mg/kg, P.O.)
- **Group IV:** EEFB (High dose 400 mg/kg, P.O.).

Each animal was placed in isolation in metabolic cages, 24 hours before commencement of the experiment for adaptation and then fasted overnight with free access to water. Urine samples were collected at the end of 5 hours after dosing. The urine samples were filtered and finally stored at 20°C for electrolyte analysis [16].

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.

Result and Discussion

The acute oral toxicity assay showed that the ethanolic extract of leaves of *F*icus benjamina *L*. extracts did not show any signs of toxicity and behavioral changes after 24 hours and 72 hours until the end in a dose of 2000mg/kg. Therefore, the extracts were safe and the doses of 200 ($1/10^{th}$) and 400 ($1/5^{th}$) were used for pharmacological studies.
EEFB at the dose of 400mg/kg and furosemide (20mg/kg) increased the urine volume significantly (**$P<0.05$, ***$p<0.001$) when compared with control rats. Based on urine volume in rats, the diuretic action of the EEFB at 200 and 400mg/kg were 1.52 and 2.2 respectively. Maximum diuretic action was observed at the dose of 400mg/kg (Table 2 and Fig 1). EEFB at 200 and 400mg/kg compared with standard furosemide, showed 65% and 82% diuretic activity (Lipschitz value), respectively (Table 2 and Fig 1).
EEFB at both the doses (200 and 400 mg/kg) and the furosemide (20 mg/kg) increased the urinary excretion of Na^+, $K^{+,}$ and Cl^- compared to control. The EEFB at the dose of 200 mg/kg does not significantly increase Na^+ and K^+ and shows a significant (*$p<0.05$) increase in urinary excretion of Cl^-. The EEFB at the dose of 400 mg/kg significantly (*$p<0.01$, *$p<0.05$) increased the urinary excretion of Na^+, $Cl^{-,}$ and K^+ respectively. The standard (furosemide) also significantly (***$p<0.001$), **$p<0.01$) increased the urinary excretion of Na^+, Cl^- and K^+ respectively (Table 3 and Fig 2, 3, 4).
The EEFB at both the doses (200 and 400 mg/kg) and the furosemide (20 mg/kg) showed potent saluretic activity. EEFB at both the doses of (200 and 400 mg/kg) and the furosemide (20 mg/kg) showed significant (*$p<0.05$, **$p<0.001$, ***p<0.0001 respectively) increase in saluretic activity when compared to the control group (Fig 5 and Table 4). A natriuretic ratio > 2.0 indicates a favorable natriuretic activity. EEFB at 400 mg/kg and furosemide at 20 mg/kg showed favorable natriuretic activity when compared to the control group (Fig 19 and Table 8). EEFB didn't show carbonic anhydrase inhibition activity in our study (Fig 7 and Table 4).

Table 1: Assessment of Acute Toxicity Studies

Group	Dose	No. of rats	Mortality	
			24hrs	72hrs
Extracted dose	5gm	6	0	0
	50gm	6	0	0
	300gm	6	0	0
	2000gm	6	0	0

Table 2: Effect of ethanolic extract of leaves of *Ficus benjamina* L. on urine volume, diuretic action, and diuretic activity

Groups	At 5hrs after the drug administration			At 24hrs after the drug administration		
	Urine volume (mL)	Diuretic action[a]	Diuretic activity[b]	Urine volume(mL)	Diuretic action[a]	Diuretic activity[b]
Control (10ml/kg)	1.00±0.17	1	_	3.40±0.27	1	_

Furosemide (20mg/kg)	2.46±0.25***	2.46	1	5.96±0.49	1.75	1
EEFB (200mg/kg)	1.52±0.24	1.52	0.61	4.78±0.37	1.40	0.80
EEFB (400 mg/kg)	2.20±0.13**	2.2	0.89	5.58±0.26	1.6	0.93

Table 3: Effect of ethanolic extract of *F*icus benjamina *L*. on urinary electrolyte concentration (Na^+, K^+ and Cl^-)

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)	88.24□5.34	55.60□4.00	120.0□10.84	1.00	1.00	1.00
Furosemide (20 mg/kg)	186.5□5.31***	86.10□7.44**	215.1□11.20***	2.11	1.54	1.79
EEFB (200 mg/kg)	116.1□16.69	67.94□3.48	173.9□10.92*	1.31	1.22	1.44
EEFB (400 mg/kg)	165.4□16.94**	78.10□5.76*	194.4□18.38**	1.87	1.40	1.62

Table 4: Effect of ethanolic extract of leaves of Ficus benjamina L. on saluretic, natriuretic, and carbonic anhydrase inhibition activity

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)	208.3□10.73	1.62□0.15	0.84□0.08	1.00	1.00	1.00
Furosemide (20mg/kg)	401.6□10.26****	2.26□0.28	0.78□0.03	1.92	1.39	0.92
EEFB (200 mg/kg)	289.9□24.45*	1.74□0.31	1.01□0.08	1.39	1.07	1.20
EEFB (400 mg/kg)	377.8□18.80****	2.22□0.42	0.82□0.04	1.81	1.37	0.97

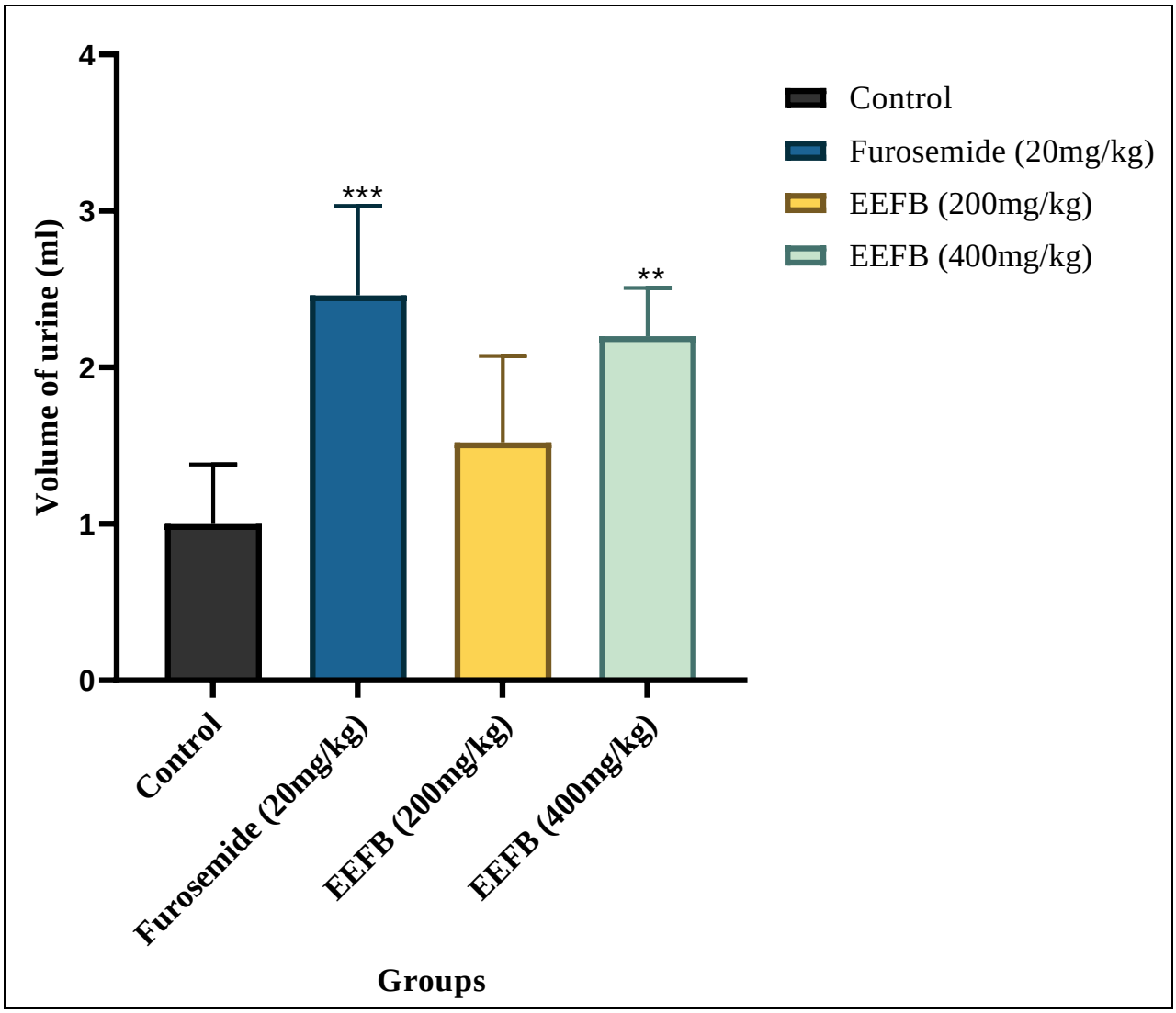

Fig 1: Effect of EEFB and furosemide on urine volume.

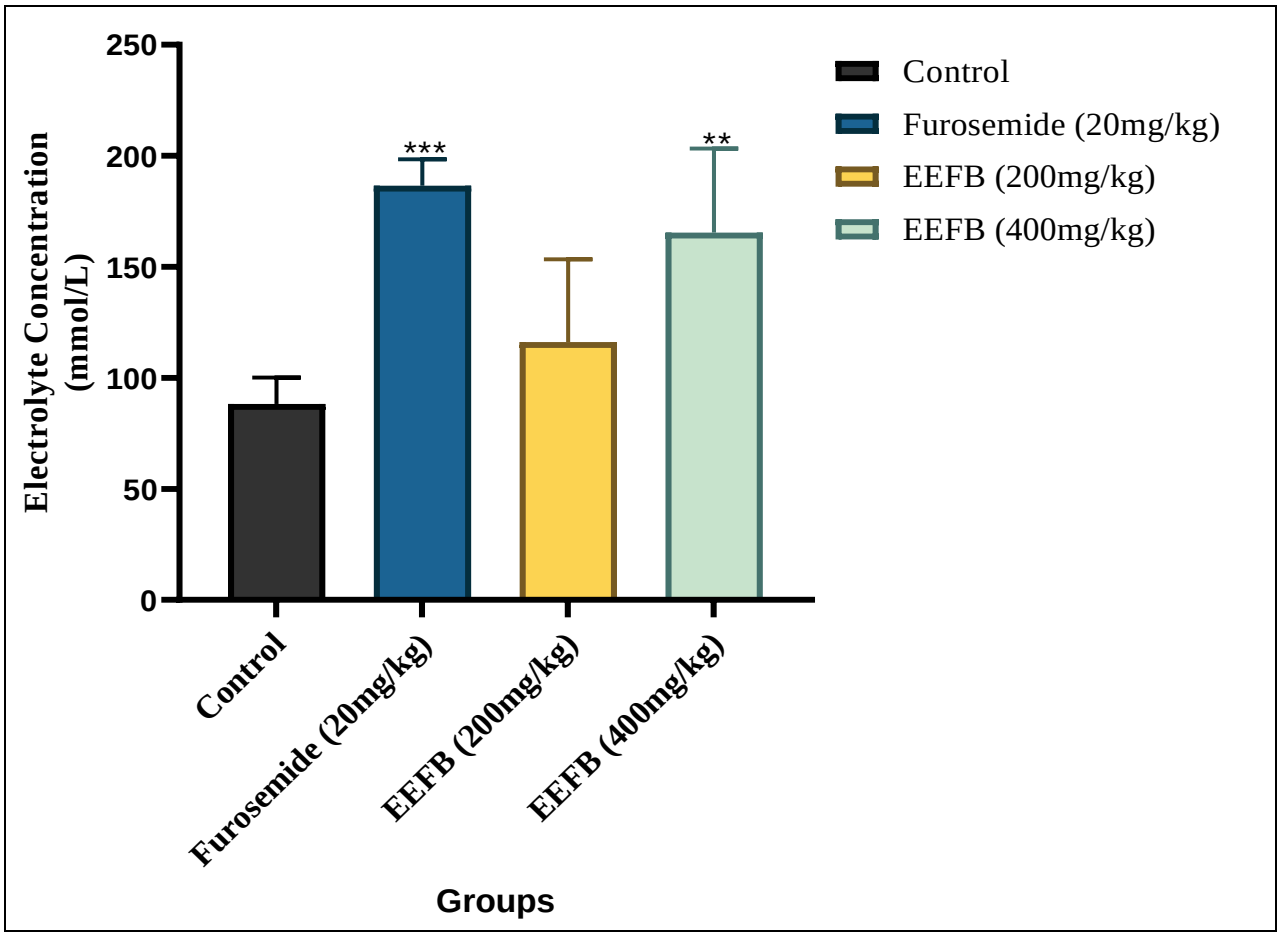

Fig 2: Effect of EEFB and furosemide on sodium

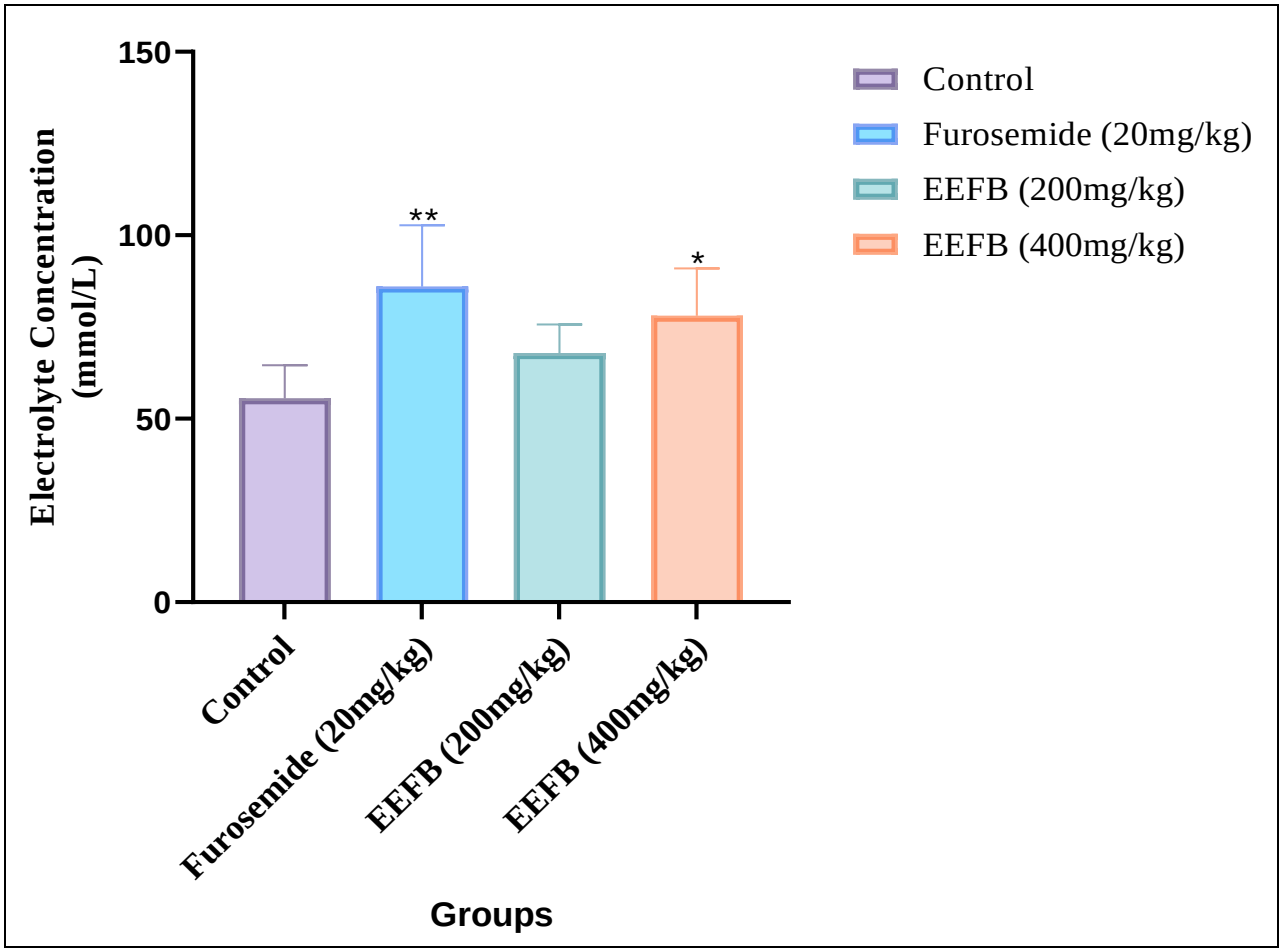

Fig 3: Effect of EEFB and furosemide on potassium

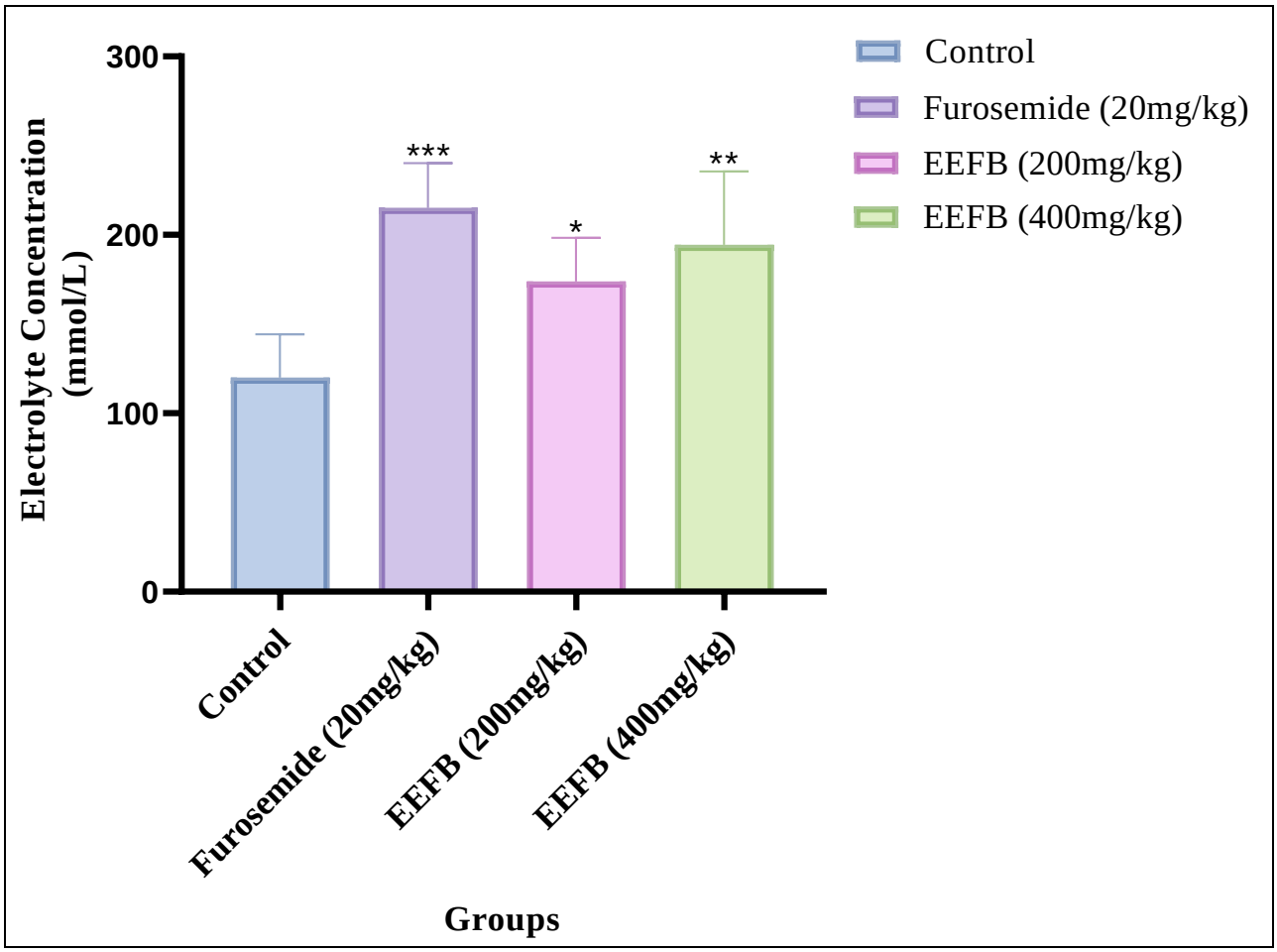

Fig 4: Effect of EEFB and furosemide on chloride.

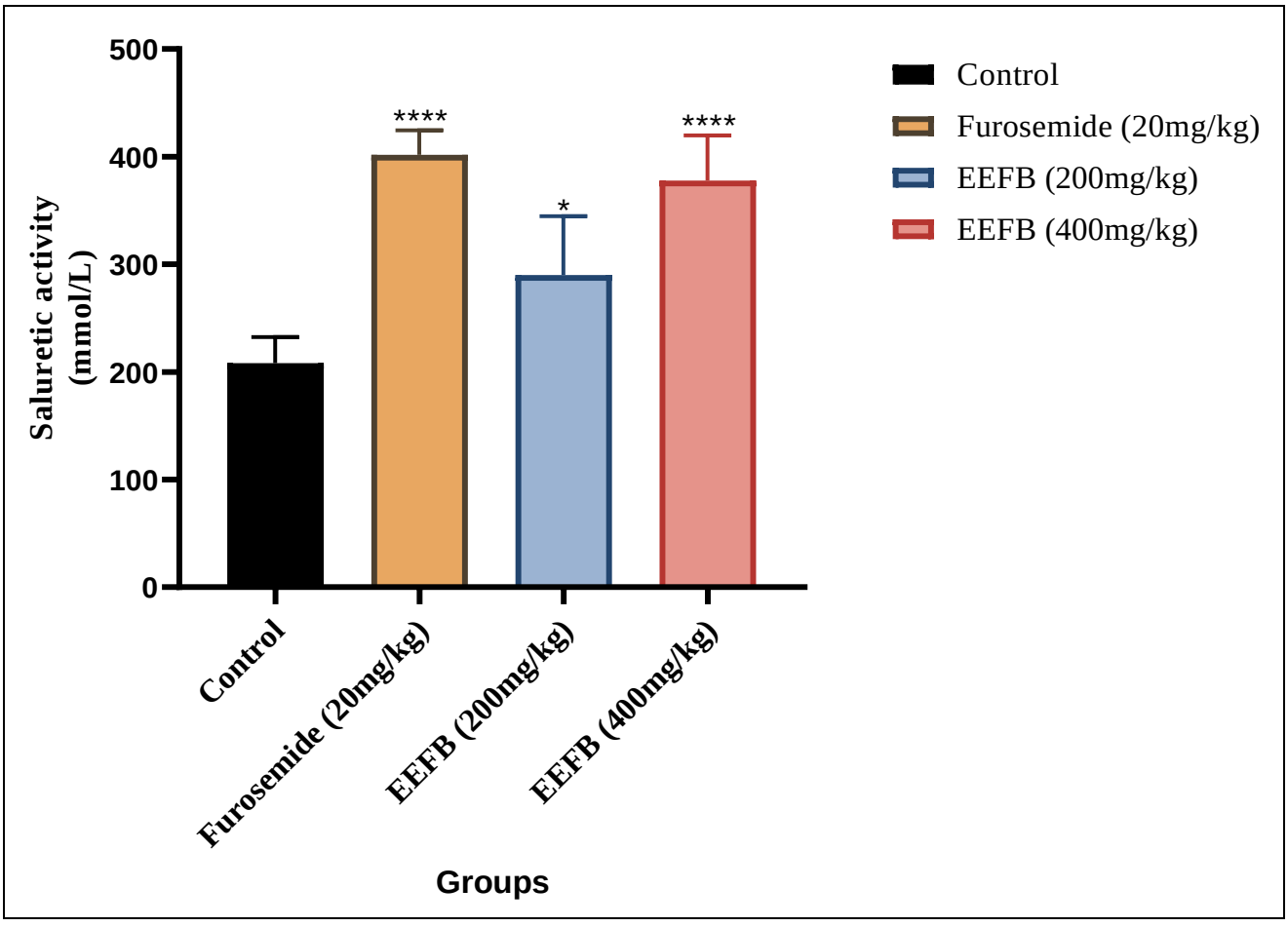

Fig 5: Effect of EEFB and furosemide on saluretic activity

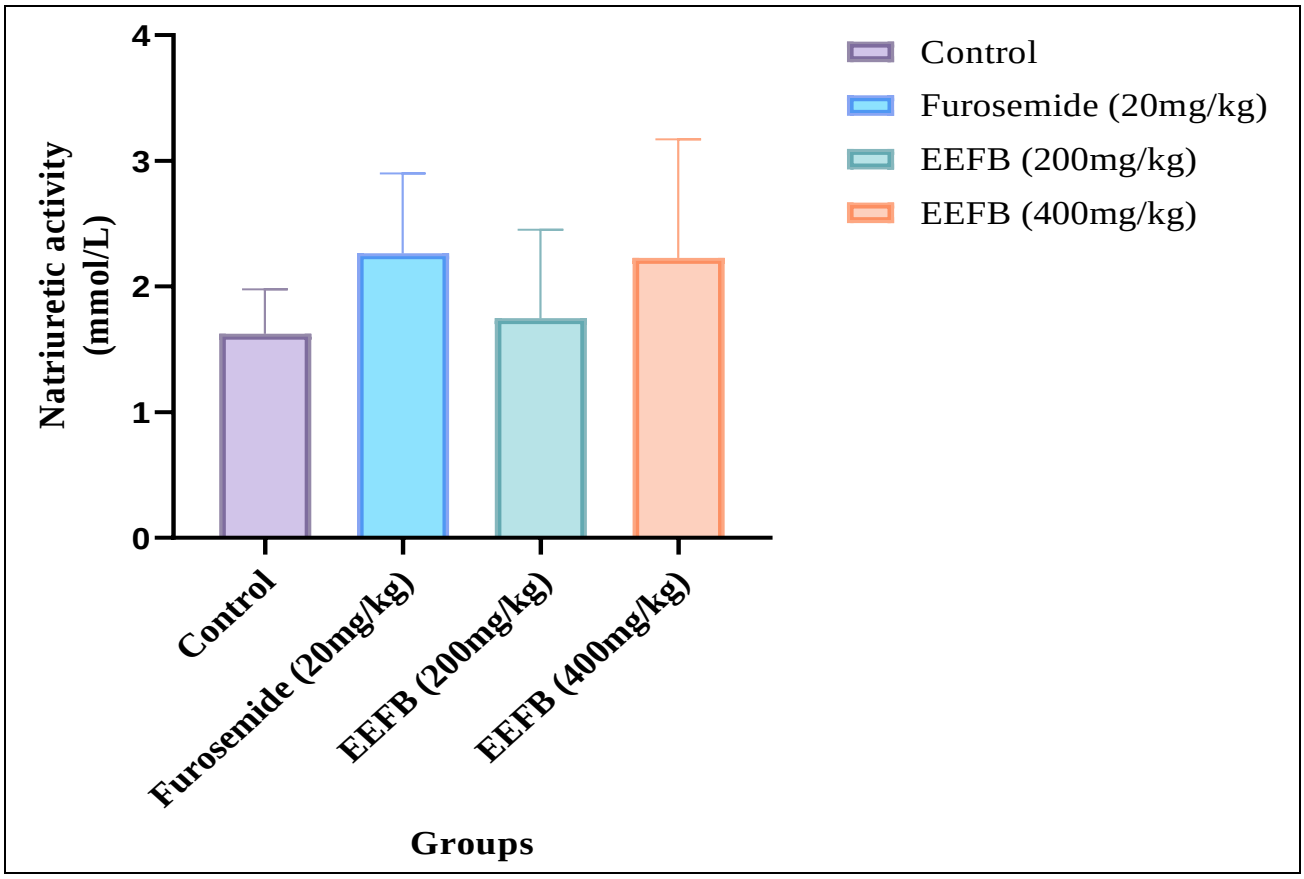

Fig 6: Effect of EEFB and furosemide on natriuretic activity

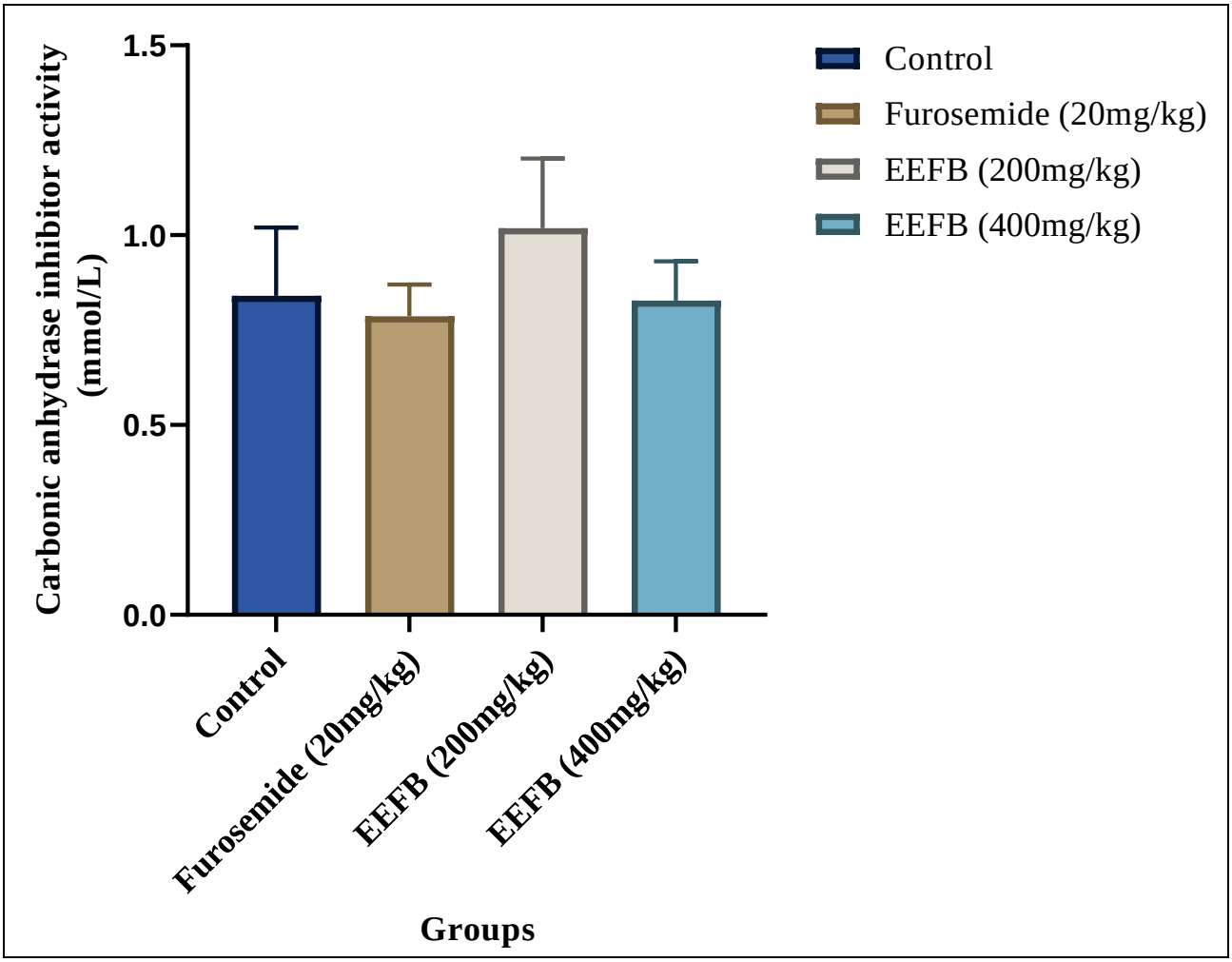

Fig 7: Effect of EEFB and furosemide on carbonic anhydrase inhibition

Diuretics are drugs that cause an increase in the urinary output of electrolytes and water from the kidney. The loss of sodium and water in the urine is due to the reabsorptive processes occurring at different segments of the nephron. The increased water loss is secondary to the increased excretion of sodium chloride. This is achieved either by directly acting on different segments of the nephron or by indirectly modifying the contents of the urinary filtrate [17].

In the present study, the diuretic effect of both doses of EEFB was evaluated in Wistar albino rats. The results indicated that EEFB at both doses showed an increase in urinary electrolyte concentration when compared to the control group and at the dose of 400 mg/kg significantly (*p<0.05, **p<0.01) increased the electrolyte concentration of potassium, sodium and chloride ions and urine volume in a dose-dependent manner when compared to the control group (Table 2 & 3 and Fig 1, 2, 3, 4). EEFB at 200 and 400 mg/kg compared with standard furosemide, showed 65% and 82% diuretic activity (Lipschitz value) respectively. EEFB at both doses showed good diuretic action when compared to the control group (Table 2).

The diuretic effect of the EEFB is indicated by the increase in both water and excretion of sodium and potassium. This effect may be produced by stimulation of initial vasodilation[18], or by producing inhibition of tubular reabsorption of water and electrolyte anions, but the result in both the cases being diuresis[19]. The increased sodium and water excretion may provide a strong basis for antihypertensive action and edema.

The EEFB at both doses (200 and 400 mg/kg) showed a significant (*p<0.05, ***p<0.0001 respectively) saluretic effect when compared to the control group (Table 4 and Fig 5). The ratio of Na^+/K^+ indicates natriuretic activity and a value greater than 2.0 indicates a favorable natriuretic effect. In the present study, EEFB at the dose of 400 mg/kg showed a value greater than 2.0 (Table 4 and Fig 6) which indicates a good natriuretic effect. The ratio of $CI^-/(Na^+ + K^+)$ is calculated for CAI and 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. In the present study EEFB at the doses 200 and 400 mg/kg did not exhibit any CAI, as the values of CAI were 1.01 and 0.82 respectively (Table 4 and Fig 7).

The EEFB at both doses resulted in a significant increase in urine volume and also an increase in urinary electrolyte concentration of (Na^+, K^+, and Cl^-) ions when compared to the control group. This effect may be due to the synergetic mechanism of the [HCO_3^-/Cl^-], [HCO_3^+/H^+] and the [Na^+/H^+] antiporter, leading to dieresis [20]. This is a characteristic of high-ceiling diuretics. Furosemide increases urine output as well as possesses saluretic activity. Furosemide belongs to the loop or high ceiling diuretics, which acts by inhibiting $Na^+/K^+/2Cl^-$ cotransport of the luminal membrane in the ascending limb of the loop of Henle and increases excretion of Na^+ and Cl^- from the body[21]. In the present study, EEFB also increased the urine volume (Table 2 and Fig 1) and excretion of urinary electrolyte concentration of Na^+, K^+, and Cl^- ion[78] (Table: 3 and Figs 2, 3, 4) which may act the same as that of furosemide.

The active principles responsible for the diuretic effects of the extracts of the plant have not yet been elucidated but preliminary phytochemical analysis of the extract of EEFB revealed the presence of polar compounds such as flavonoids. Earlier researchers showed that flavonoids, saponins, and organic acids would be responsible for the diuretic effect of the plant [22].

Conclusion

The ethanolic extract of leaves of *Ficus benjamina L*. did not show any symptom of a change in behavior or mortality up to 2000 mg/kg of oral dose which indicates the therapeutic safety of the pharmacologically active doses. In the present investigation, the yield of ethanolic extract of leaves of *Ficus benjamina L*. was 20%. Phytochemical analysis revealed the presence of tannins, carbohydrates, phytosterols, flavonoids, terpenoids, phenolics, oils, fats, and saponins in ethanolic extract of leaves of *Ficus benjamina L*. The EEFB showed significant diuretic activity by increasing the urine output and urinary electrolyte concentration of Na^+, K^+, and Cl^- ions showing good diuretic action and diuretic activity (Lipschitz value). EEFB also showed highly significant saluretic activity slightly good natriuretic activity and absence of carbonic anhydrase inhibition (CAI) when compared to the control group. The diuretic activity of EEFB might be due to the presence of phytochemicals like tannins, flavonoids, and terpenoids. The present study confirms the folklore use of leaves of *Ficus benjamina L*. Furthur studies are required for the isolation and characterization of the active compounds and to determine their mode of action.

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