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Research Article

POTENT AND EFFICACIOUS DIURETIC ACTIVITY WITH POTASSIUM-SPARING EFFECT OF *TERMINALIA BELERICA* FRUIT PULP AQUEOUS EXTRACT IN WISTAR ALBINO RATS

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ABSTRACT

Objective: Diuretics are commonly used in edematous conditions, but they carry a high risk of adverse effects which varies from electrolyte imbalance to fatal hypokalemia which is a matter of concern. Many herbal drugs possess diuretic effect used as folklore medicine for various ailments found to be safer. Hence, our present study aim to evaluate potency and efficacy of *Terminalia belerica* fruit pulp aqueous extract (TBFPAE) in Wistar albino rats as a diuretic.

Methods: Wistar albino rats weighing 200-250 g of either sex were divided into five groups containing six animals each. Sodium chloride solution (0.9%) was given to all the animals as a priming dose. Group I received normal saline (20 ml/kg, orally) which served as control, Group II served as standard and received frusemide (10 mg/kg, orally), whereas Groups III, IV, and V received TBFPAE in the dose of 9, 18, and 36 mg/kg orally, respectively, and were placed individually in metabolic cage. The urine volume and electrolyte concentrations of Na^+, K^+, and Cl^- in the urine were determined at the end of 5^{th} hr.

Results: Our study revealed that TBFPAE possesses diuretic effect with a significant potassium-sparing effect comparable to frusemide in the dose of 9, 18, and 36 mg/kg in Wistar albino rats.

Conclusion: TBFPAE possesses diuretic effect with a significant potassium-sparing effect comparable to frusemide in the dose of 9, 18, and 36 mg/kg in Wistar albino rats comparable to frusemide.

Keywords: Aqueous extract, Diuretic activity, Fruit pulp, Potassium-sparing effect, *Terminalia belerica.*

INTRODUCTION

Diuretics are commonly used in edematous conditions and are also generally prescribed for the treatment of hypertension as a first line drug but also as an adjuvant for congestive heart failure, glaucoma, diabetes insipidus, and liver ailments. Sulfanilamide was the first diuretic discovered in 1949. There are many diuretics available to the physicians which are divided into four groups based on the site of action on nephrons in the kidney. Loop diuretics acts on ascending limb of the loop of Henle with the efficacious diuretic effect but is short acting, and they include frusemide, ethacrynic acid, and bumetanide. Thiazides are moderately efficacious diuretics, and they include hydrochlorothiazide and hydroflumethiazide. On the contrary, diuretics such as triamterene, amiloride, and spironolactone have only a weak diuretic effect with potassium-sparing effect. The fourth group consists of substances which function as vasodilator or osmotic agents [1].

Edema formation in heart failure is clearly established where diuretics are primarily used to reduce plasma volume. Similarly, diuretics are also being increasingly used in the treatment of non-edematous states such as hypertension, diabetes insipidus, and hypercalciuria. Electrolyte imbalance leading to hypokalemia, hyponatremia, hyperkalemia, and/or hyperuricemia relates to its adverse effects [2].

Terminalia belerica, belongs to the family - Combretaceae and commonly known as myrobalan, is a deciduous tree found throughout the Indian forests and plains. The tree grows to a height of about 30-40 m and 2-3 m in girth. The stem is straight, and the leaves are broadly elliptical in shape and are clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 cm in diameter is gray to dark brown. Pulp of the fruit extract is used as they have properties of astringent, antiseptic, rejuvenative, brain tonic, expectorant, and laxative. It is used for coughs and sore throat. It is also used in dysentery, diarrhea, liver disorders, leprosy, fever, and hair care. *T. belerica* is reported to promote digestive power, wound healing and is curative of ulcers, local swelling, anemia, diabetes, and chronic recurrent fever. The fruits of *T. belerica* are purgative, laxative, and gastroprotective and are used to alleviate asthma, piles, and cough. *T. belerica* has been reported to exhibit a variety of biological activities such as antidiabetic anticancer antimutagenic and antiviral activity. The aqueous extract of *T. belerica* fruit pulp contains total phenolic and tannin content which is said to have good antioxidant activities also [3-11].

METHODS

Institutional Animal Ethics Committee (Ref. No. IAEC/02//2013/CPCSEA) approval was obtained on October 05, 2013, before conducting the study. Committee for the Purpose of Control and Supervision of Experiment on Animals guidelines was strictly followed. The test drug was obtained from Sri Lakshmi Ayurvedic Dispensary, Central Market, Mangalore and Dr. Krishna Kumar. G, Chairman, Department of Applied Botany, Mangalore University, Mangalore, Karnataka, India, authenticated *T. belerica* fruit.

Procedure

Around 1000 g of air and shade dried crude powder of *T. belerica* fruit pulp was extracted with distilled water in soxhlet extractor for about 36 hrs. It was again dried and reduced under controlled pressure and temperature (40-50°C) by rotator evaporator. A brownish mass was obtained weighing 145 g. This yield was 14.5% w/w with respect to its dried powder.

Either sex of Wistar albino rats weighing 200-250 g were obtained from animal house of A. J. Institute of Medical Sciences and Research Centre,

Table 1: Effect of TBFPAE on urine electrolytes and volume

Groups	Sodium (m. mol/L)	Potassium (m. mol/L)	Chloride (m. mol/L)	Volume (ml)
Control (1% normal saline), 20 ml/kg, per oral	81.66±0.84	47±0.57	107.16±0.47	5.33±0.21
Standard (frusemide 10 mg/kg, per oral)	124.16±0.60**	86.6±0.61**	148.5±0.76**	14.33±0.42**
TBFPAE 9 mg/kg, per oral	115±1.34**	21.8±1.74**	175±1.06**	9.5±0.42**
TBFPAE 18 mg/kg, per oral	114.5±1.20**	16.83±0.79**	148.83±1.01**	12.16±0.47**
TBFPAE 36 mg/kg, per oral	115.66±0.84**	33.33±0.71**	134.8±1.19**	12±0.57**

n=6, Observations are mean±SEM, ANOVA followed by Dunnet's multiple comparison test. **p<0.01: Highly significant, TBFPAE: *Terminalia belerica* fruit pulp aqueous extract, SEM: Standard error of mean, ANOVA: Analysis of variance

Mangalore, Karnataka, India. All animals were housed at 24±2°C with a 12:12 hrs light and dark cycle. All animals had free access to food and were provided water *ad-libitum*. All animals were acclimatized to the experimental lab for 7 days before the study. Animals were divided randomly into five groups of six animals in each. All the animals received priming dose of 0.9% sodium chloride solution (20 ml/kg, orally). Group I received vehicle saline (20 ml/kg, orally) which served as control and Group II received the standard drug frusemide (10 mg/kg, orally) and served as standard. Groups III, IV, and V received doses of *Terminalia beleria* fruit pulp aqueous extract (TBFPAE) in the dose 9, 18, and 36 mg/kg orally, respectively, which was suspended in normal saline. Each animal was placed in an individual metabolic cage which separates feces and urine at room temperature. Urine was collected at the end of 5th hr after dose administration. Concentration Na^+, K^+, and Cl^- in the urine were determined by suitable biochemical methods and expressed in terms of mmol/L, whereas urine volume was expressed in a milliliter. Flame photometer was used to determine Na^+ and K^+ concentrations. Cl^- concentration in the urine was estimated by titrating it with silver nitrate solution (N/50) using 3-5 drops of 5% potassium chromate as an indicator. The Na^+/K^+ concentration ratio in the urine at the end of 5th hr was calculated to assess the diuretic potential of TBFPAE [12-14].

Statistics

ANOVA followed by Dunnet's multiple comparison test. $p<0.05$ is considered as significant.

RESULTS

In our study, we found that TBFPAE showed significant diuretic activity which is comparable to frusemide in the dose of 9, 18, and 36 mg, respectively (Table 1, Figs. 1 and 2) with a p <0.01 .

DISCUSSION

Diuretics are one of the first-line antihypertensive medications. However, diuretics have its own limitations in the modern system of medicine-like hypokalemia which is found with the use of frusemide and thiazide group of diuretics. Moreover, polypharmacy is another area of concern with the use of above said diuretics as potassium-sparing diuretics has to be commonly combined to prevent hypokalemia, but it also has its own adverse effects as well such as hyperkalemia and gynecomastia (spironolactone). *T. belerica* has been mentioned to possess antihypertensive effect [15]. In view of this, we have tried to evaluate its role as antihypertensive by screening diuretic activity. In our study, we found that TBFPAE showed significant diuretic activity which is comparable to frusemide in the dose of 9, 18, and 36 mg, respectively (Table 1, Figs. 1 and 2). When compared to frusemide, TBFPAE has increased the excretion of volume and sodium in urine with potassium-sparing effect which is an added advantage over modern diuretics and also contributes to prevent polypharmacy-related adverse effect. It is also found that TBFPAE is almost equally potent and efficacious diuretic comparable to frusemide. We opine that thiazide would be a better drug to compare with the diuretic activity of TBFPAE as it is most commonly used in hypertension as a diuretic. However, further studies are needed to evaluate its exact molecular mechanism of action as diuretic by comparing with frusemide and thiazide as well.

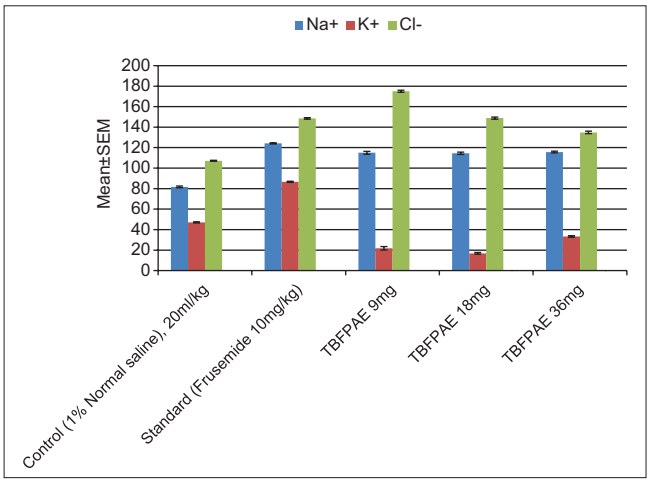

Fig. 1: Effect of *Terminalia belerica* fruit pulp aqueous extract on urine electrolytes in Wistar albino rats

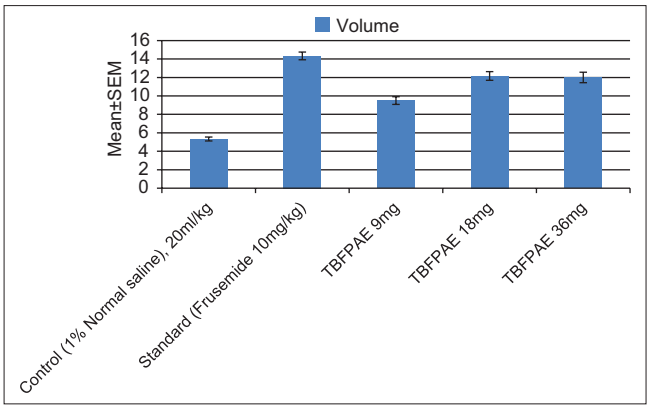

Fig. 2: Effect of *Terminalia belerica* fruit pulp aqueous extract on urine volume in Wistar albino rats

CONCLUSION

Our study revealed that TBFPAE possesses diuretic effect with a significant potassium-sparing effect comparable to frusemide in the dose of 9, 18, and 36 mg/kg in Wistar albino rats.

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