Journal of Scientific and Innovative Research 2023; 12(2): 20-26
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2023; 12(2): 20-26

Received: 31-12-2022
Accepted: 23-04-2023

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Phytochemical Investigation and Exploration of the Diuretic Activity of Ethanolic Extract of Flowers of *Jasminum grandiflorum* L. as Traditional Medicine in Wistar Albino Rats

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Abstract

The entire plant of Jasminum grandiflorum L. is said to have potent diuretic properties in Indian traditional medicine. However, this plant's potential as a diuretic has not yet been studied. Therefore, the objective of the current study was to assess the diuretic activity of an ethanolic extract of J. grandiflorum flowers in Wistar albino rats. In acute oral toxicity studies, the extract was shown to be safe up to a dose level of 5000 mg/kg body weight. The diuretic effect of the EEJG was evaluated by various parameters such as urine volume, urinary electrolytes (Na^+, K^+, and Cl^-), saluretic effects, natriuretic effects, and CAI (carbonic anhydrase inhibitory) at two different doses (500 and 1000 mg/kg) in Wistar albino rats. Furosemide was used as a reference drug. The urine volume was measured at 5 h and 24 h, and electrolyte excretion (Na^+, K^+, and Cl^-) at a 24 h duration was measured. The urine output at both doses (500 and 1000 mg/kg) was significantly (*$p < 0.05$ and **$p < 0.01$, respectively) increased at 5 h and 24 h, and urinary electrolyte excretion was significantly (*$p < 0.05$) increased at 1000 mg/kg when compared to the control. EEJG also showed a significant (**$p < 0.01$) saluretic and good natriuretic effect at 1000 mg/kg. The previous studies showed that flavonoids, terpenoids, and saponins are responsible for the diuretic activity. The present study also showed the presence of flavonoids, terpenoids, and saponins, which might be responsible for the diuretic activity of J. grandiflorum. Further studies are required to isolate the active ingredient from the extract, which is responsible for the diuretic activity of the extract.

Keywords: *Jasminum grandiflorum* L.; Diuretic activity; Furosemide; Saluretic; Natriuretic; Carbonic anhydrase.

INTRODUCTION

Plants have been important and basic to the preventive and curative health care system since time immemorial, and the use of indigenous herbal medicine is a very ancient art and an integral part of treatment [1]. Diuretics are medications that accelerate the kidney's excretion of water and electrolytes through the urine. The net loss of water and sodium in the urine is because of one or more reabsorptive processes that take place at various segments of the nephron. The increase in water loss is actually secondary to the increased excretion of sodium chloride. This is accomplished either by directly acting on certain nephron segments or by indirectly altering the components of the urine filtrate [2].

Diuretics are used to treat several conditions, such as hypertension, heart failure, influenza, liver cirrhosis, and some renal disorders. Arterial hypertension is generally among the most frequent pathologies in elderly patients worldwide. This pathology raises more concerns as it increases the major risk for cardiovascular accidents. In such cases, diuretics are given for treatment [3]. In cases of poisoning or overdose, the use of some diuretics is also advised to aid in the patient's body excreting more of the poisonous or overdosed material. Some people with eating disorders may abuse diuretics to help them lose weight. The availability of diuretics also had a major impact on the understanding of renal physiology, but they were associated with severe side effects such as hypokalaemia, ototoxicity, hyperglycemia, erectile dysfunction, hypersensitivity reactions, hyponatraemia, etc [2]. Nowadays, the world is paying attention to 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 those of synthetic drugs, but the probability of adverse symptoms is minimal. Medicinal herbs are the most significant source of diuretics. Many researchers demonstrated that the studies of herbal plants used in traditional medicines

that are used as diuretics have shown progressive improvement in recent decades and could be a valuable tool in human pathology treatment [3]. There are 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].

Jasminum grandiflorum Linn. (*Oleaceae*) is frequently referred to as jasmine. It is a glabrous, twining shrub with a high therapeutic value. It is a native of Asia, including Kashmir, Afghanistan, Persia, the Nilgiris, France, Italy, China, Japan, India, Morocco, and Egypt [5]. The flower is acrid and bitter, with a sharp taste. It is effective in treating dental and oral conditions, particularly toothaches. They are also beneficial to women when brewed as a tonic because they help prevent breast cancer and stop uterine bleeding [6]. It is widely used in Ayurveda for the treatment of various ailments, including chronic constipation, flatulence, dysmenorrhea, amenorrhea, ringworm, skin diseases, ulcers, giddiness, and diabetes [7]. The plant is reported to possess spasmolytic, anti-inflammatory, anti-microbial, antioxidant, anti-ulcer, cytoprotective, chemopreventive, wound healing, and anti-acne activities [8]. The present study was undertaken to investigate the diuretic activity of an ethanolic flower extract of *J. grandiflorum*.

MATERIALS AND METHODS

Preparation of plant extract

J. grandiflorum flower powder (100 g) was defatted with 150 ml of petroleum ether and extracted with ethanol by the 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

Wistar albino rats of either sex (150–250 g) were obtained from Mahaveer Enterprises in Hyderabad, Telangana, India (1656/PO/Bt/S/12/CPCSEA). They were kept in a HKES MTRIPS animal house with a 12-hour light and 12-hour dark cycle at a temperature of 25 ± 1°C and a relative humidity of 45% to 55%. The animals had free access to food pellets and unlimited access to water. The experiments were conducted with prior approval from the IAEC (IAEC approval no. HKES/MTRIPS/IAEC/122/2021-22).

Chemicals

Ethanol LR and petroleum ether 60-80°C LR (SDFCL, Lower Parel, Mumbai, India). Furosemide Tablet I.P. Lasix 40 mg (Sanofi, Ankleshwar, Gujarat, India).

Acute oral toxicity test

Acute oral toxicity tests were carried out in accordance with OECD (Organization for Economic Cooperation and Development) guideline 425 [9]. Healthy adult Swiss mice of either sex (20–25 g) were used for the study. Before the extract was given orally, food but not water was withheld for four hours. All extracts were given in a graduated dose manner, starting with a dose of 175 mg/kg, p.o. When no abnormality or death was observed, the next doses of 550, 1750, and 5000 mg/kg were selected. At the dose of 5000 mg/kg, an additional four mice were dosed. All the animals were monitored for initially 30 minutes and then 24 hours for behavioral, neurological, and autonomic profiles, and also observed for any lethality or death over the next 48 hours.

Preliminary phytochemical screening

An ethanolic extract of the flowers of *J. grandiflorum* was qualitatively tested for the detection of alkaloids, saponins, terpenoids, tannins, flavonoids, and steroids [10].

Diuretic activity

All the animals were randomly divided into four groups of six rats each: a control group, a furosemide-treated group, and two EEJG-treated groups. The control group was treated with 10 ml/kg of body weight of normal saline, the furosemide-treated group was treated with furosemide 20 mg/kg [2]. The EEJG-treated group was subdivided into two subgroups and treated with 500 and 1000 mg/kg doses of ethanolic extract of *J. grandiflorum* (EEJG), respectively. All the doses were administered orally.

After all the treatment administration, each animal was placed in metabolic cages. 24 hours prior to the experiment, all animals were fasted overnight with free access to water. Urine samples were collected after 5 hours and 24 hours from the last dose. The urine samples were filtered and finally stored at 20°C for electrolyte analyses [2, 11].

Measurement of urine parameters

Total urine volume was measured after 5 and 24 hours for all the animals. To estimate the total concentrations of electrolytes (Na^+, K^+, and Cl^-) in urine, the total urine output samples (over 24 h) were determined by the Ion Selective Electrode method described in the instruction manual of the biochemical kits (Central Diagnostics Pvt. Ltd., Mumbai, Maharashtra, India).

Diuretic action and activity (Lipschitz value)

The diuretic action and diuretic activity (Lipschitz value) were derived from the ratio of urine volume in the test group and that in the control and furosemide groups, respectively. Before the experiment began, it was decided that diuretic activity would be regarded as "moderate" and "good" if the values were between 0.72–1.00 and 1.00–1.5 [12].

Saluretic, natriuretic, and carbonic anhydrase inhibition (CAI)

Saluretic activity was measured by adding the urinary excretion of sodium and chloride. Natriuretic activity was determined by calculating the ratio Na^+/K^+ (values greater than 2.0 indicate a favourable natriuretic effect). To estimate carbonic anhydrase inhibition, the ratio $Cl^-/(Na^+ + K^+)$ was calculated (CAI can be excluded at ratios between 1.0 and 0.8; with decreasing ratios, slight to strong CAI can be assumed) [12].

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 behavioural or autonomic abnormalities, as well as no mortality, were observed in any of the mice treated with the ethanolic extract of *J. grandiflorum* up to the dose 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 ethanolic extract from flowers of *J. grandiflorum* was 8%. The preliminary phytochemical assessment of EEJG showed the presence of alkaloids, saponins, terpenoids, flavonoids, and steroids (Table 1).

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

The details of urine volume, diuretic action, and diuretic activity are presented in Table 2. The results revealed that *J. grandiflorum* exhibited dose-dependent diuretic activity at 5 h and 24 h (Figure 1 & 2). The total urine volume over the period of 5 h and 24 h was measured for the ethanolic extract of *J. grandiflorum*, the furosemide, and the control

group. EEJG at both the doses (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 with control rats. On the basis of urine volume in rats, the diuretic action of the EEJG at 500 and 1000 mg/kg was 2.28 & 2.61 at 5 h and 1.53 & 1.76 at 24 h, respectively. The diuretic activity (Lipschitz value) of *J. grandiflorum* exhibits moderate diuretic activity.

Effects on urinary electrolyte excretion

EEJG at both doses (500 and 1000 mg/kg) increased the urinary excretion of Na^+, K^+, and Cl^- compared to control; the extract response was dose-dependent (Figure 3, 4 & 5). The EEJG at a dose of 1000 mg/kg significantly (*$p < 0.05$) increased the urine excretion of Na^+ and K^+ (Table 3).

Effects on saluretic, natriuretic, and carbonic anhydrase inhibition (CAI)

The EEJG at both doses (500 and 1000 mg/kg) showed potent saluretic activity, but only at 1000 mg/kg did it show a significantly (**$p < 0.01$) higher level of saluretic activity when compared to the control group (Table 4 & Figure 6). Natriuretic ratio > 2.0 indicates favourable natriuretic activity. EEJG at 1000 mg/kg showed favourable natriuretic activity (Table 4 & Figure 7). EEJG at both doses (500 and 1000 mg/kg) didn't show any carbonic anhydrase inhibition, as the values of the CAI ratio were 0.92 and 0.79, respectively (Table 4 & Figure 8).

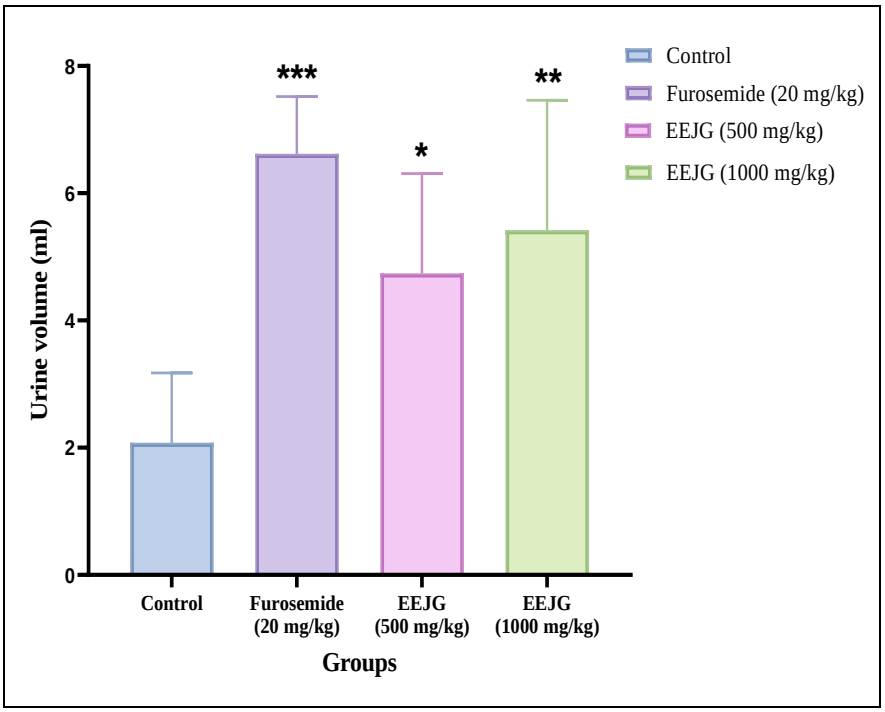

Figure 1: Effect of EEJG and furosemide on urine volume at 5h

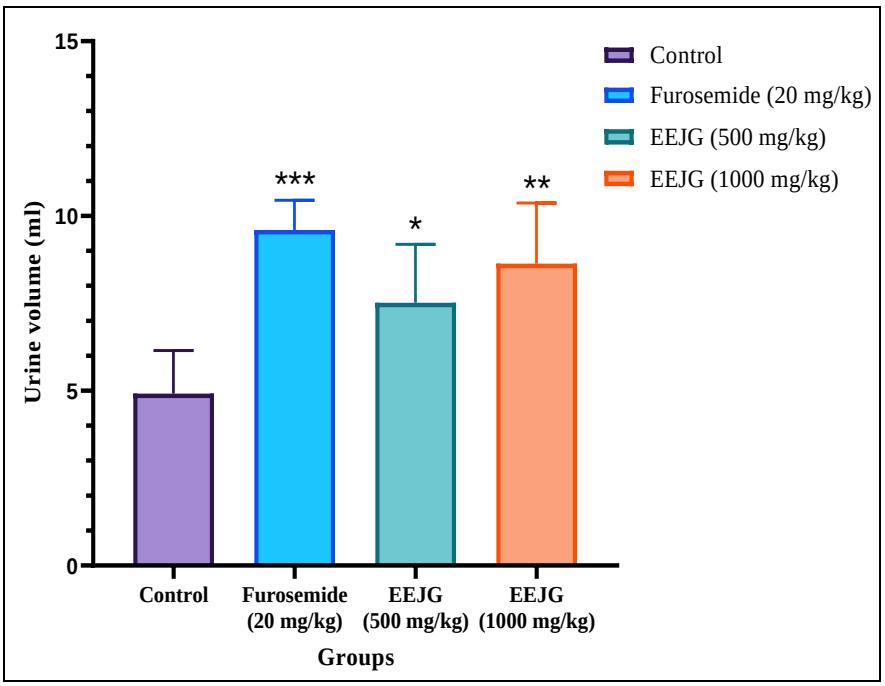

Figure 2: Effect of EEJG and furosemide on urine volume at 24 h

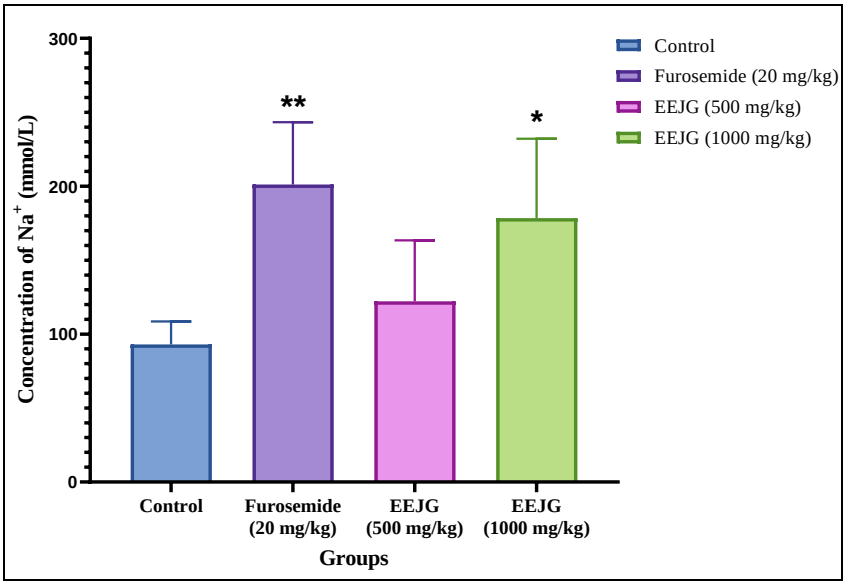

Figure 3: Effect of EEJG and furosemide on urinary sodium

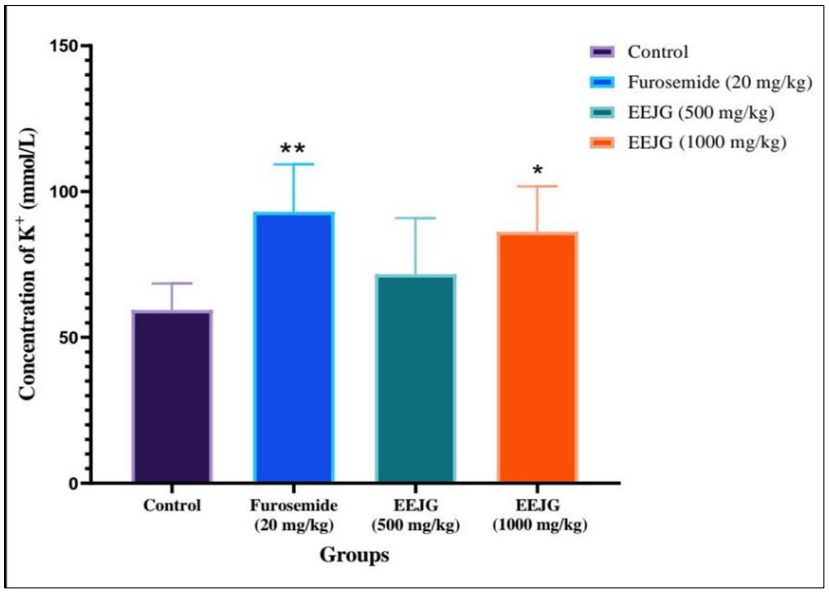

Figure 4: Effect of EEJG and furosemide on urinary potassium

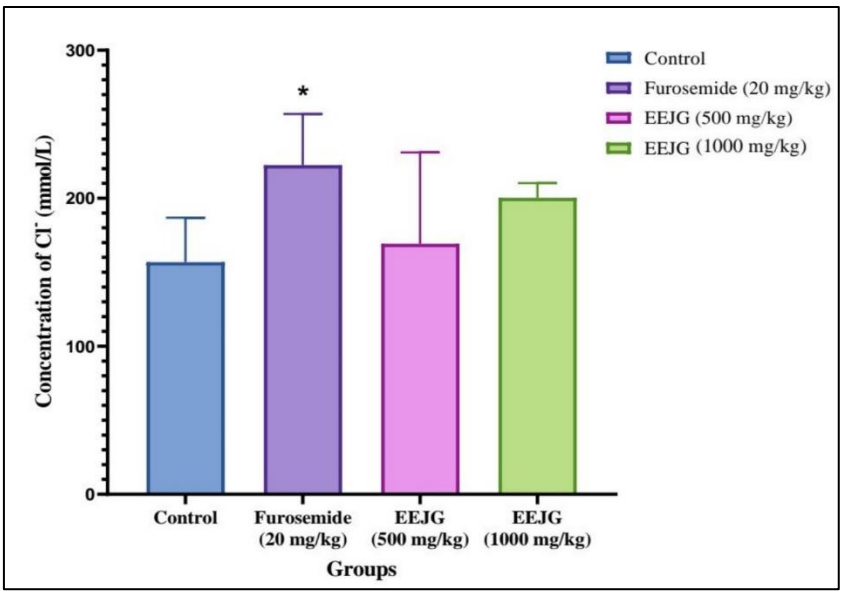

Figure 5: Effect of EEJG and furosemide on urinary chloride

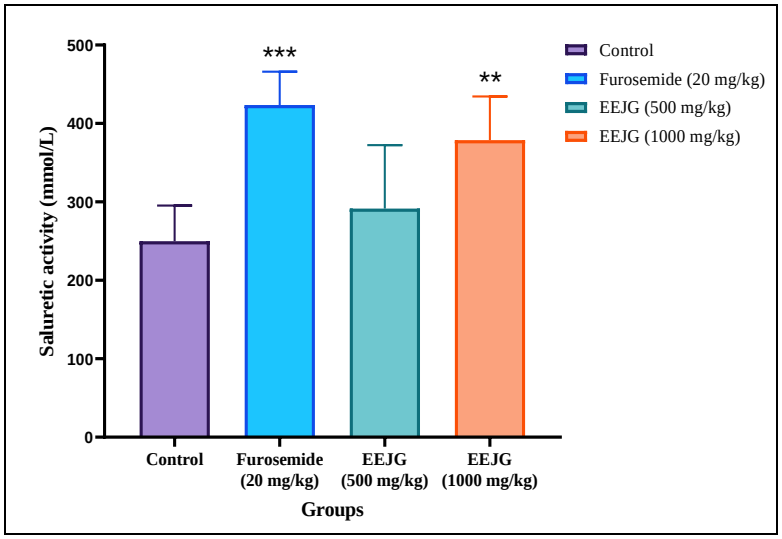

Figure 6: Effect of EEJG and furosemide on saluretic activity

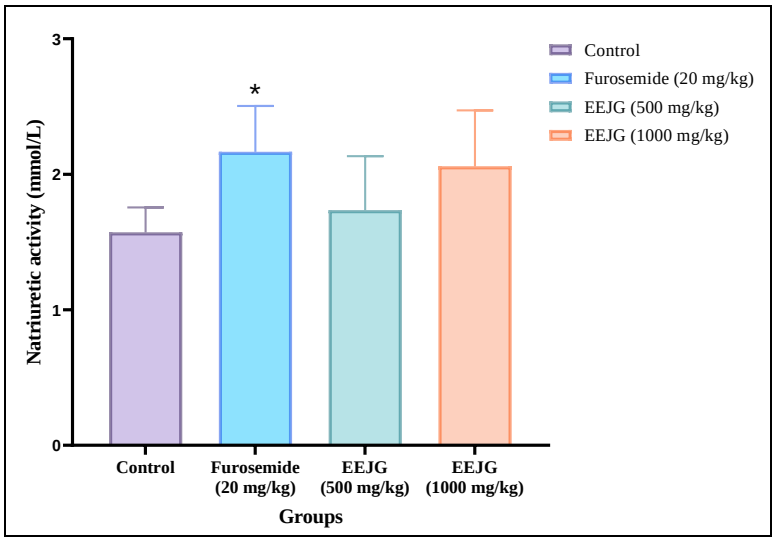

Figure 7: Effect of EEJG and furosemide on natriuretic activity

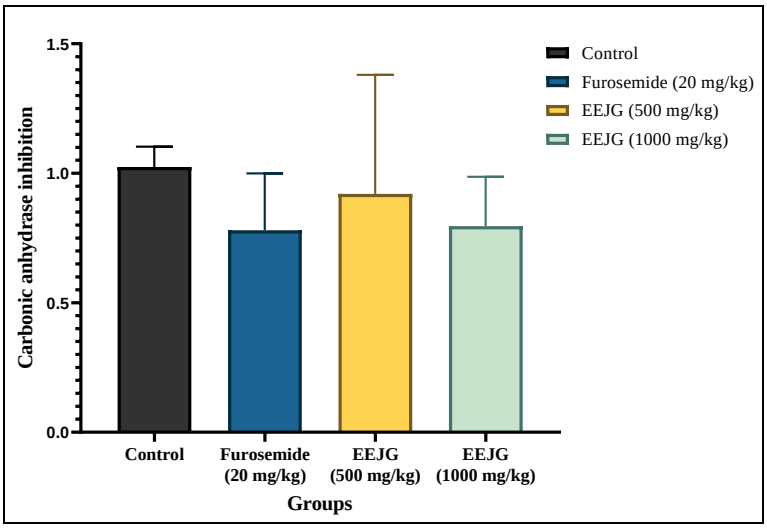

Figure 8: Effect of EEJG and furosemide on carbonic anhydrase inhibition

Table 1: Chemical constituents present in ethanolic extract of *Jasminum grandiflorum* Linn. flowers

TEST	ETHANOLIC EXTRACT
Alkaloids	+
Saponins	+
Terpenoids	+
Tannins	-
Flavonoids	+
Steroids	+

(+) - Positive, (–) - Negative

Table 2: Effect of ethanolic extract of flowers of *Jasminum grandiflorum* Linn. on urine volume 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)	2.08±0.49	1.00	——	4.92±0.55	1.00	——
Furosemide (20 mg/kg)	6.62±0.41***	3.18	1.00	9.60±0.38***	1.95	1.00
EEJG (500 mg/kg)	4.74±0.70*	2.28	0.72	7.52±0.75*	1.53	0.78
EEJG (1000 mg/kg)	5.42±0.91**	2.61	0.82	8.64±0.77**	1.76	0.90

n=6; Values are expressed as mean ± S.E.M; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control group.

EEJG – Ethanolic extract of *Jasminum grandiflorum* 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 flowers of *Jasminum grandiflorum* Linn. 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)	93.00±6.99	59.40±4.06	156.8±13.47	1.00	1.00	1.00
Furosemide (20 mg/kg)	201.2±18.80**	93.06±7.27**	222.4±15.45*	2.16	1.57	1.42
EEJG (500 mg/kg)	122.2±18.46	71.63±8.62	169.3±27.61	1.31	1.21	1.08
EEJG (1000 mg/kg)	178.5±24.02*	86.20±6.96*	200.3±4.46	1.92	1.45	1.28

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 flowers of *Jasminum grandiflorum* Linn. 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)	249.8±20.33	1.57±0.08	1.02±0.04	1.00	1.00	1.00
Furosemide (20 mg/kg)	423.6±18.91***	2.17±0.15*	0.78±0.10	1.70	1.38	0.76
EEJG (500 mg/kg)	291.4±36.19	1.74±0.18	0.92±0.21	1.17	1.11	0.90
EEJG (1000 mg/kg)	378.8±24.95**	2.06±0.18	0.79±0.08	1.52	1.31	0.7

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

DISCUSSION

Diuretics are medications that increase the rate of urine output and sodium excretion. They are used to adjust the volume and composition of body fluids in various clinical situations, such as high blood pressure, heart failure, cirrhosis, and nephritic syndrome [13].

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

EEJG at a dose of 1000 mg/kg showed a significant (**$p < 0.01$) 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 [12]. Na^+/K^+, indicates that more Na^+ is excreted than K^+, which is seen as a highly positive profile for diuretics. In the present study, EEJG 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 $CI^-/(Na^+ + K^+)$ 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 [12]. In the present study, EEJG at 500 and 1000 mg/kg doses did not exhibit any CAI, as the values of the CAI ratio were 0.92 and 0.79, respectively (Table 4).

In light of the patterns of urine output and electrolyte (Na^+, K^+ and Cl^-) excretion, as well as the diverse bioactive principles present in the crude extract, it appears that the action of the extracts may be caused by the synergistic mechanism involving the [$HCO3^-/Cl^-$], [$HCO3^+/H^+$], and the [Na^+/H^+] antiporters, which results in diuresis [11]. Furosemide can increase the urinary output and also possess saluretic activity [13]. Furosemide is a loop or high-ceiling diuretic that works by inhibiting $Na^+/K^+/2Cl^-$ cotransport of the luminal membrane in the ascending limb of the loop of Henle and increasing Na^+ and Cl^- excretion from the body [13]. In the present study, EEJG also increased urine volume (Table 2) and excretion of urinary Na^+, K^+, and Cl^- (Table 3) and may act in the same way as furosemide.

The precise nature of the active principle causing the observed effects of EEJG is unknown, but it's possible that secondary metabolites (flavonoids, terpenoids, and saponins) are responsible for diuretic activity by exerting favourable effects on kidney physiological processes, such as increasing potassium sparing capacity, interacting with the adenosine A1 receptor linked to diuretic activity, or possibly inhibiting tubular reabsorption of water and accompanying anions [14]. EEJG contains flavonoids, terpenoids, and saponins, which may be responsible for the plant's diuretic effect by increasing the rate of glomerular filtration, which in turn promotes increased urine formation [15].

CONCLUSION

This study confirms the significant diuretic activity of the ethanolic extract of flowers of *J. grandiflorum*, which might be due to flavonoids, terpenoids, and saponins present in the extract as phytoconstituents. This study supports the plant's traditional use as a diuretic. However, further research is required to fractionate and isolate the molecule from the extract, and studies are also required to be carried out to know the exact mechanism of the fraction for the diuretic activity of the molecule.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B: Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research. 2010;3:91-100.
2. Jayanthi MK, Siddamma A: To Evaluate the Diuretic Activity in Ethanolic Extract of Leaves of *Delonix Regia* in Wistar Albino Rats. Biomedical & Pharmacology Journal. 2018;11(2):959-963.
3. Martin N, Pantoja C, Chiang L, Bardisa L, Araya C, Roman R: Hemodynamic effects of a boiling water dialysate of maize silk in normotensive anaesthetised dogs. J. Ethnopharmacology. 1991;31:259-262.
4. Sameer AH, Dr. Mitali MB: Advantages of natural diuretics over synthetic diuretics as a part of treatment. World journal of pharmacy and pharmaceutical sciences. 2019;8(3):310-327.
5. Sandeep S, Padmaa P: *Jasminum grandiflorum* Linn (Chameli): Ethnobotany, Phytochemistry and Pharmacology – A review. Pharmacologyonline. 2009;2:586-595.
6. Dr. Shivananda NB, Krishna M: Influence of ethanolic extract of *Jasminum grandiflorum* Linn. flower on Wound healing activity in rats. Indian Journal of Physiology and Pharmacology. 2007;51(2):189-194.
7. Shiva KS, Nagalakshmi NC, Santhosh K, Yogesh HS: Hypoglycemic Activity of Ethanol Extract of *Jasminum Grandiflorum* Flowers in Vivo and Cytotoxicity of Its Chloroform Isolate in Vitro. International journal of diabetes and metabolic disorders. 2018;3(2):1-9.
8. Patil SM, Saini R: Anticonvulsant Activity of Methanolic Extract of *Jasminum grandflorum* Linn in Experimental Animals. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012;3(2):43.
9. Committee for the purpose of control and supervision of Experimental Animals (CPCSEA), OECD Guidelines for the testing of Chemicals, revised draft guidelines 425(#26), Acute oral toxicity-Acute toxic class method, revised document, India: Ministry of Social Justice and Empowerment, 2008.
10. Ghani A: Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Dhaka, Bangladesh; 2003, 331-332.
11. Fahad IA, Mohd NA: Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats. Saudi Journal of Biological Sciences. 2016;23:749-753.
12. Vogel HG: Drug discovery and evaluation: pharmacological assays. 2nd ed. Germany: Springer; 2002;1:323-5.
13. Ashok KD, Senthilkumar GP, Thamil SV, Mazumder UK, Gupta M, Ray SK: Study on diuretic activity and electrolytes excretion of methanolic extract of *Lippia nodiflora (Verbenaceae)* in rats. Oriental Pharmacy and Experimental Medicine. 2008;8(1):39-46.
14. Majid A, Najam US, Naveed A, Hafiz MA, Jamshaid M, Sabira S, Kamran B: Phytochemical Screening and Evaluation of the Diuretic Activity of Aqueous Methanol Extract from Aerial Parts of *Mentha viridis* Linn (*Labiatae*) in Albino Rats. Tropical Journal of Pharmaceutical Research. 2014;13(7):1121-1125.
15. Sara T, Ephrem E, Akeberegn GA, Chala B. Evaluation of the diuretic activity of aqueous and 80% methanol extracts of Croton macrostachyus (Euphobiaceae) leaves in saline-loaded rats. Journal of Experimental Pharmacology. 2021, 213-221.