

Original Research Article

Aqueous Extract of *Flueggea leucopyrus* Increases Urine Output in Rats

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Abstract

Purpose: To investigate the effect of *Flueggea leucopyrus* Wild aqueous extract (FLAE) on the urinary output of rats.

Method: Three different doses of FLAE (500, 1000 and 1500 mgkg⁻¹), furosemide (13 mg kg⁻¹ as diuretic reference) and distilled water (as control) were orally administered to healthy adult hydrated rats. Cumulative urine output was monitored hourly over 6 h. Selected urinary parameters were determined for 1500 mg kg⁻¹ dose, furosemide, and water-treated groups to investigate the possible mode of action. Using these data, standard urine indices were calculated. Glomerular filtration rate (GFR) in terms of creatinine clearance, overt toxicity, renal toxicity, liver toxicity, as well as phytochemical screening were also determined.

Results: The highest dose (1500 mgkg⁻¹) significantly increased urine output (control vs. treated: 0.74 ± 0.07 vs. 1.38 ± 0.09 mL/100 g) ($p < 0.05$) $r^2 = 0.925$). The effect of FLAE was dose-dependent. Increase in urine output was observed from the 1st hour, peaked at 2nd hour and lasted till the 6th hour. Furthermore, 1500 mgkg⁻¹ dose of FLAE caused a significant ($p < 0.05$) increase in urinary K⁺ level, aldosterone secretion index, thiazide secretion index and GFR at 24 h. However, significant decrease in urinary Na⁺ level (control vs. treated: 7915.2 ± 423.1 vs. 6611.2 ± 181.3 ppm) was noted with the highest dose ($p < 0.05$). Serum alanine transaminase (ALT), serum aspartate transaminase (AST) and urea levels were not altered significantly ($p > 0.05$). However, serum creatinine level was elevated significantly ($p < 0.05$). Phytochemical screening showed that FLAE contains primary, secondary, tertiary, quaternary alkaloids/amine oxides, triterpenoids, unsaturated sterols, leucoanthocyanins, tannins of pyrogallol type and cyanogenic glycoside.

Conclusion: The results show that FLAE exhibits moderate oral aquaretic activity.

Keywords: *Flueggea leucopyrus*, Diuretic, Aquaretic, Urine output, Toxicity, Phytochemical

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INTRODUCTION

Flueggea leucopyrus Wild (Katupila in Sinhala, Irubulai, Mudbulanji in Tamil) is a large bush with long straggling thorny branches and small globose white berries. It is distributed in Sri Lanka, India and Burma [1]. *F. leucopyrus* is a medicinal plant used for the treatment of many diseases including cancer in the Ayurvedic

system of medicine [2]. It is claimed that the juice of the *F. leucopyrus* leaves is used to destroy maggots in sores [1]. According to the Indian reports, it is claimed that the plant is sweet, cooling, diuretic, aphrodisiac and tonic, and is useful in vitiated conditions of pitta, burning sensation, strangury, seminal weakness and general debility [3]. The leaves act as a disinfectant and its paste is used by Indian

people to extract any extraneous materials from body tissues without surgery [3]. Leaves are boiled and taken twice a day for stomach aches [4]. The leaves are also used in the treatment of piles and fibroids [5]. The roots are used in the treatment of testicular enlargement and in the cure of edema. The whole plant is used for the cure of cancer in the sole of the foot [5]. It is also used in the treatment of abdominal lumps and liver hypertrophy and portal hypertension. The bark of stem is used for tooth ache [5].

The di(phenyl)-(2,4,6,-trinitrophenyl)iminoazanium (DPPH) free radical scavenging activity of the aqueous extract of the leaves and stem has been reported [2]. The aqueous extract of the leaves show concentration dependent nitric oxide NO radical scavenging activity at concentrations less than 5 µg mL⁻¹. The aqueous extract of the stem does not show any dose dependent relationship towards NO radical scavenging activity [2]. Securinine and its stereoisomers viroallosecurinine which are alkaloids extracted from *F. leucopyrus* possesses antibacterial activity against *E. coli*, *S. aureus*, *M. smegmatis*, and *P. aeruginosa* [6]. It is also reported that securinine exhibited antifungal activity against many kinds of pathogenic and saprophytic fungi [6]. Although the diuretic activity of *F. leucopyrus* is claimed by Indian reports, it has not been scientifically tested and validated.

This study was undertaken to investigate the urine output of *F. leucopyrus* whole plant using its aqueous extract and conscious hydrated rats.

EXPERIMENTAL

Drugs and chemicals

Furosemide was obtained from State Pharmaceutical Corporation, Colombo, Sri Lanka, while all the other chemicals used in phytochemical analysis were manufactured by BDH Chemicals, Poole, England.

Animals

Healthy adult albino rats with average weight (200 - 225 g) were used (n = 6 per group). They were housed in standard environmental conditions. The animals were fed with pelleted foods (Ceylon Grain Elevators, Colombo, Sri Lanka) and clear drinking water. Except at the time of experimental procedure, the animals were handled only during cage cleaning. All the experiments were conducted in accordance with the internationally accepted laboratory animal

use and care and guidance [7] and rules of the Faculty of Medicine, University of Colombo, Sri Lanka. For animal experimentations, ethical clearance was obtained (EC/11/137) from Ethics Review Committee of Faculty of Medicine, University of Colombo.

Plant collection

Whole plant of *F. leucopyrus* were collected from Wijerama (Latitude: 6.911 °N Longitude: 79.87 °E), Hambantota (Latitude: 6.24 °N Longitude: 81.07 °E), Kohuwela (Latitude: 6.86 °N Longitude: 79.89 °E) in Sri Lanka in November 2011. Identification and authentication were done at Department of Plant Science, University of Colombo, by Dr Sudheera Ranwala. Voucher specimen was deposited (wdr/SAD/15) in the museum of Department of Zoology, University of Colombo.

Preparation of *Flueggea leucopyrus* aqueous extract (FLAE)

The whole plant was washed under running water and air dried for four weeks to get a constant weight and cut into small pieces. The pieces were refluxed with water for 16 h in a round bottom flask and fitted with a Leibig condenser (1050 g of plant material in 15 L of water). The brownish red solution was filtered using a sintered funnel and concentrated up to 3 L using a heating mantle. The concentrated sample was freeze-dried, and stored in air tight bottles at (-20 °C). The freeze-dried powder was dissolved in distilled water to prepare the required doses [8-10].

Evaluation of extract electrolyte profile

Aqueous extracts of plant materials collected from three different areas in Sri Lanka (Wijerama, Hambantota and Kohuwela) were subjected to several experiments to evaluate the Na⁺ content, K⁺ content, pH, conductivity and total dissolved solids TDS of each sample.

Urine analysis

Na⁺ and K⁺ contents were determined using atomic absorption spectrophotometer (FAAS, GBC 935 plus, GBC Scientific Equipment pvt. Ltd, Victoria, Australia) while pH was measured using a pH meter (Eutech, pH 510, Eutech Instruments, Singapore). Conductivity and total dissolved solids were determined using conductivity meter (Jenway, 4510, Bibby Scientific Ltd, UK)

Evaluation of urine output

Thirty rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails [11]. Each of these rats was then orally administered with 15 mL of isotonic saline (NaCl, 0.9 % w/v) to impose a uniform water load. 45 min later, these rats were randomly divided into five equal groups (n = 6 per group) and treated orally in the following manner, (Group 1) 1 mL of 500 mgkg⁻¹ of FLAE; (Group 2) 1 mL of 1000 mgkg⁻¹ of FLAE; (Group 3) 1 mL of 1500 mgkg⁻¹ of FLAE; (Group 4) 1 mL of distilled water; (Group 5) 1 mL of 13 mgkg⁻¹ of furosemide. Each of these rats was individually placed in metabolic cages and cumulative urine output was determined at hourly intervals over 6 h. To investigate the possible mode of action, the urine collected from group III (1500 mgkg⁻¹) and group IV (water control) were subjected to further investigations [11-14].

Determination of creatinine clearance

Twelve rats were randomly divided into two equal groups (n = 6 per group), fasted and hydrated as described previously. One group was orally administered with 1 mL of distilled water and the other with 1500 mgkg⁻¹ of FLAE. These rats were individually placed in metabolic cages and their cumulative urine outputs were measured after 24 h. Blood was collected from tails using aseptic precautions at 24 h post treatment. Serum was separated and creatinine levels in the serum and urine were determined using Randox kits. Creatinine clearance was computed as per instructions given by the manufacturer using data obtained. Creatinine clearance was taken as an estimation of the glomerular filtration rate [11-14].

Sub chronic toxicity studies

Twelve rats were randomly divided into two equal groups (n = 6 per group). One group was orally daily administered with the highest dose (1500 mgkg⁻¹) and the other group with 1 mL of distilled water consecutively for 30 days. During this period, each rat was observed for the following toxicity signs; overt signs of toxicity (salivation, lachrymation, breathing distress, ptosis, stupor, squint, teeth exposure, writhing, convulsion, tremors, yellowing of fur and loss of fur), stress (erection of fur and exophthalmia), behavioral abnormalities (such as impairment of spontaneous movements, climbing, cleaning of face, ataxia, rolling and other postural changes) and aversive behaviors (biting and scratching, licking of tail and paw, intense grooming or vocalization) and diarrhea.

On day 1 post treatment (31st day), these rats were anaesthetized with ether and blood was drawn from tails using aseptic precautions. Examination of serum urea and creatinine (to examine renal toxicity), alanine transaminase (ALT) and aspartate transaminase (AST) (to examine liver toxicity), were made using respective kits [8-14].

Phytochemical screening

The crude aqueous extract of plant *F. leucopyrus* was subjected to phytochemical screening as described by Fransworth [15].

Statistical analysis

Data are represented as Mean ± SEM. Statistical comparisons were made by Mann-Whitney U-test and Student's t-test using Minitab 14.1 version statistical package. Dose dependence was determined using Pearson's correlation test. Significant level was set at $p < 0.05$.

RESULTS

As shown in Table 1, the highest dose (1500 mgkg⁻¹) of FLAE and furosemide (13 mg kg⁻¹) significantly ($p < 0.05$) increased the cumulative urine output for 6 h. The regression analysis revealed that this effect was dose-dependent ($r^2 = 0.925$, $p < 0.05$). The urine output started to increase at 1 h and peaked at 2 h. (control vs treated: 0.22 ± 0.05 vs 0.94 ± 0.09).

As shown in Table 2, the highest dose (1500 mgkg⁻¹) of FLAE significantly ($p < 0.05$) increased urinary potassium level. On the other hand, the urinary sodium level was significantly ($p < 0.05$) reduced in the treated group. Of the indices calculated, aldosterone secretion index and thiazide secretion index were significantly ($p < 0.05$) reduced.

The highest dose of FLAE significantly ($p < 0.05$) increased the glomerular filtration rate at 24 h. (control vs treated: 1.26 ± 0.20 vs 13.04 ± 1.90 mL h⁻¹).

Table 3 shows the variation of Na⁺, K⁺, pH, TDS (total dissolved solids) and conductivity of FLAE samples collected from Wijerama, Hambantota and Kohuwala in Sri Lanka. These parameters did not vary significantly ($p < 0.05$) according to the site of sample collection.

In the toxicity study, no morbidity and mortality were observed in the rats of treated and control

groups during treatment. The highest dose of FLAE did not induce any overt signs of toxicity, stress, behavioral aberrations or aversive behavior.

As depicted in Table 4, serum AST, ALT and urea levels investigated were not altered significantly. However, serum creatinine level was significantly elevated ($p < 0.05$).

DISCUSSION

This study was undertaken for the first time to examine the effect of *F. leucopyrus* aqueous extract whole plant on the urine output of hydrated rats. The results showed that *F. leucopyrus* plant possesses moderate aquaretic (in terms of cumulative urine output and urinary

Table 1: Cumulative urine output of rats over a 6-hour period following oral administration of FLAE

Treatment	Cumulative urine output (mL/100 g)						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
1 mL of distilled water(control)	0.02 ± 0.01	0.08 ± 0.03	0.22 ± 0.05	0.31 ± 0.05	0.44 ± 0.06	0.63 ± 0.08	0.74 ± 0.07
500 mg kg ⁻¹ HWE	0.07 ± 0.07	0.17 ± 0.16	0.43 ± 0.21	0.46 ± 0.23	0.50 ± 0.22	0.66 ± 0.22	0.83 ± 0.16
1000 mg kg ⁻¹ HWE	0.18 ± 0.08	0.29 ± 0.19	0.33 ± 0.19	0.48 ± 0.34	0.48 ± 0.34	0.84 ± 0.32	0.92 ± 0.28
1500 mg kg ⁻¹ HWE	0.05 ± 0.02	0.46 ± 0.08*	0.94 ± 0.09*	1.06 ± 0.11*	1.21 ± 0.11*	1.26 ± 0.18*	1.38 ± 0.09*
13 mg kg ⁻¹ Furosemide	0.15 ± 0.06	1.34 ± 0.31*	1.87 ± 0.51*	2.16 ± 0.55*	2.34 ± 0.64*	2.42 ± 0.7*	2.67 ± 0.59*

* $p < 0.05$, compared with control; mean ± SEM (n = 6)

Table 2: Effect of orally administered FLAE (1500 mgkg⁻¹) on some selected urine parameters of rats

Parameter	Control group 1 mL of H ₂ O	Treated group 1500 mg kg ⁻¹
Na ⁺ (ppm)	7915.7 ± 423.1	6611.2 ± 181.3*
K ⁺ (ppm)	3518.0 ± 310.0	5203.9 ± 240.4*
Na ⁻ (ppm)	79.7 ± 7.3	61.1 ± 7.6
Ca ²⁺ (ppm)	258.6 ± 28.7	205.5 ± 28.1
Mg ²⁺ (ppm)	12096.1 ± 636.1	12348.4 ± 348.1
HCO ₃ ⁻ (ppm)	0.02 ± 0.004	0.01 ± 0.002
pH	6.63 ± 0.14	6.68 ± 0.16
Aldosterone secretion index	2.45 ± 0.17	1.31 ± 0.06*
Thiazide secretion index	0.65 ± 0.01	0.54 ± 0.01*
Carbonic anhydrase index	1.07 ± 0.008	1.05 ± 0.005
Aquaretic action	1.0	1.81
Aquaretic potency	1.0	0.23
Sodium saluretic index	1.0	0.84
Potassium saluretic index	1.0	1.48
Magnesium saluretic index	1.0	0.79
Calcium saluretic index	1.0	0.77
Chloride saluretic index	1.0	1.02
Bicarbonate saluretic index	1.0	0.75

* $P < 0.05$ as compared with control; (mean ± SEM, n = 6)

Table 3: Electrolyte profile (mean ± SEM) of HWE from various areas of Sri Lanka

Parameter	Site of collection		
	Wijerama	Hambantota	Kohuwala
Na (ppm)	238.70 ± 26.70	133 ± 16.8	802.67 ± 133.7
K (ppm)	5404.30 ± 301.80	5173.7 ± 347.3	6637.67 ± 13.4
pH	5.06 ± 0.14	4.6 ± 0.01	4.88 ± 0.03
TDS (mg/L)	19.90 ± 0.16	27.9 ± 3.3	52.0 ± 8.0
Conductivity (µS)	33.20 ± 0.39	43.17 ± 2.5	72.6 ± 3.5

* $P < 0.05$ as compared with each site; TDS - Total Dissolved Solids

Table 4: The effect of oral administration (30 days) of FLAE (1500 mgkg⁻¹) on some serum biochemical parameters of rats

Parameter	Control group 1 mL of DW	Treated group 1500 mgkg ⁻¹ of HWE
AST (U/L)	63.2 ±3.2	55.6 ± 8.5
ALT (U/L)	60.8 ±11.1	60.1 ± 13.5
Urea (mg/dL)	49.7 ± 2.4	56.7 ± 5.2
Creatinine (mg/dL)	14.4 ±1.1	24.6 ±1.8*

**p* < 0.05 as compared with control (Mann-Whitney, U-test and Student *t*-Test); (mean ± SEM) *n* = 6

Table 5: Phytochemical profile of FLAE

Phytochemical	Result
Primary alkaloids	+
Secondary alkaloids	+
Tertiary alkaloids	+
Quaternary alkaloids/amine oxides	+
Triterpenoids	+
Unsaturated sterols	+
Leucoanthocyanins	+
Tannins of pyrogallol type	+
Cyanogenic glycoside	+

electrolyte concentrations) activity. For the highest dose the urine output started to increase at 1 h which was extremely rapid and lasted throughout the study (up to 6 h). This action profile indicates rapid absorption and slow metabolism or clearance which is therapeutically desirable. Further, the increase of urine output induced by FLAE was dose dependent suggesting that the effect was genuinely intrinsic and casual, and results are not due to nonspecific actions [11]. Moreover, analysis of FLAE revealed a high potassium level compared with sodium level irrespective of the site of collection of the plant. Because of this high potassium content in FLAE, it is possible that potassium overloading takes place and the kidney tubules are incapable of absorbing this excess potassium and this will lead to increase in the urinary excretion.

Even though the increase of urine output was quick and strong, urine was not hypernatremic (in terms of urinary Na⁺ level and sodium saluretic index). Further, urinary Mg²⁺, Ca²⁺, Cl⁻ levels were not significantly altered. These observations collectively suggest that FLAE is not acting as a loop diuretic. Loop diuretics cause increase in urinary Na⁺, K⁺ and Cl⁻ levels by inhibiting the Na⁺/K⁺/2Cl⁻ co-transporter in the thick region of the ascending limb of loop of Henle [16]. Even though urinary K⁺ content of treated group was increased significantly, it is unlikely be due to the loop diuretic type of action.

The FLAE did not increase aldosterone secretory index (Na⁺/K⁺) of urine suggesting that the increased urine output of FLAE is different from potassium sparing diuretics. Potassium sparing diuretics act on the distal tubule of the loop of Henle by antagonising the aldosterone hormone and increasing the Na⁺/K⁺ ratio [17].

The effect of FLAE on urine output is unlikely to be due to thiazide type mode of action, since there was a decrease of urinary thiazide diuretic index (Na⁺/Cl⁻), and impairment of urinary Na⁺ level. Simultaneously, urinary Mg²⁺ and Ca²⁺ levels decreased but the effect was not significant. Thiazide diuretics inhibit the Na⁺/Cl⁻ symporter (cotransport system) in the distal convoluted tubule by competing with the Cl⁻ binding site and increasing the excretion of Na⁺ by inhibiting the Na⁺ reabsorption. Moreover, thiazide diuretics increase urinary Mg²⁺ level while reducing Ca²⁺ level [16].

On the other hand, FLAE did not alter urinary HCO₃⁻ excretion and carbonic anhydrase index. Thus, carbonic anhydrase inhibitor diuretic type of action is unlikely to be operative with FLAE. Carbonic anhydrase inhibitors impair the carbonic anhydrase enzyme at the proximal convoluted tubule and, increase the volume of urine by preventing bicarbonate reabsorption [17].

In the present study, FLAE failed to show an increase in urinary electrolytes (in terms of Na⁺, Ca²⁺, Mg²⁺, H⁺, HCO₃⁻ levels, aldosterone secretion index and thiazide secretion index,) in spite of marked and significant increase in urine volume. On the contrary, the urine was hyponatremic (in terms of urinary Na⁺ level, sodium saluretic index). These findings indicate that the increase in urine output may be due to an aquaretic action of FLAE rather than a true diuretic effect [18]. This notion is further supported by the increased GFR of treated group in the study [19]. Aquaretics mediates their action by increasing the volume of urine via enhanced blood flow in the kidneys, thereby raising the glomerular filtration rate [19]. They do

not retard the reabsorption of Na⁺ and Cl⁻ in the renal tubules and will excrete solute free water from the body. The arginine vasopressin (AVP) or ADH is a cyclic nonapeptide neurohormone. AVP is synthesized by the hypothalamus and stored in the posterior pituitary. There are three sub-types of AVP receptors located in different tissues of the body which are known as V1a, V1b, and V2. Of these, V2 receptors are mainly found in the collecting tubules of kidney and are involved in free water reabsorption [20,21]. Aquaretics antagonize AVP effect by acting via V2 receptors and thereby retard the free water absorption [22]. Currently synthetic aquaretics such as tolvaptan, lixivaptan and conivaptan and natural aquaretics: *Solidaginis virgaurea herba*, *Betulae folium*, *Ononidis radix*, *Graminis rhizoma* are therapeutically used [19,22]. Interestingly, diuretics cannot be used as a treatment for hyponatremia. On the other hand, aquaretics are suitable to use in hyponatremia [23].

According to the phytochemical screening, FLAE was rich with primary alkaloids, secondary alkaloids, tertiary alkaloids and quaternary alkaloids/amine oxides. Apart from that, it contained triterpenoids, unsaturated sterols, leucoanthocyanins, tannins of pyrogallol type and cyanogenic glycoside. Of these phytoconstituents, only tannins have been reported to increase the volume of urine by promoting blood flow to the kidneys thereby raising the glomerular filtration rate [24]. Therefore, it is likely that the aquaretic activity of FLAE is mediated via tannins.

The results of the sub-chronic toxicity test revealed that FLAE had no adverse side effects in terms of overt signs of toxicity, stress, behavioral abnormalities, diarrhea, aversive behaviors, renal toxicity (in terms of urea levels) or liver toxicity (in terms of serum alanine transaminase and aspartate transaminase levels). However, serum creatinine level was elevated.

CONCLUSION

The results obtained in this work show that FLAE exhibits moderate oral aquaretic activity, suggesting the therapeutic potential of the plant.

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