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Research Article
Evaluation of Diuretic Effect of the Hot Water Extract of Standardized *Tragia involucrata* Linn., in Rats

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Abstract

Background: Medicinal plants have become a significant source of diuretics due to the increased side effects of diuretic drugs used in modern medicine practice. *Tragia involucrata* Linn. (Family: Euphorbiaceae) is such a source as it is being used as a diuretic in traditional medicine. **Objective:** To evaluate the diuretic activity of hot water extract of *T. involucrata* (TWE) made out of the whole plant. **Materials and Methods:** Different concentrations of TWE (550, 1100, 1650 and 2200 mg kg^{-1}), distilled water, furosemide (13 mg kg^{-1}) were orally administered (N = 6) to fasted (18 h) male Wistar rats and the urine output was monitored hourly for 5 h. Sodium and potassium content, pH, specific gravity of urine from TWE treated rats were compared with that of the controls. Preliminary phytochemical screening and standardization parameters were also evaluated in *T. involucrata*. **Results:** Urine output was significantly ($p<0.05$) increased when increasing the dose of TWE and maximum diuresis was observed at a dose of 1650 mg kg^{-1}. Onset of diuresis was within 1 h and showed maximum activity and diuretic activity declined with time. Rats treated with 1650 and 2200 mg kg^{-1} doses of TWE were significantly ($p<0.05$) higher than that of the reference drug at 1 h. Furthermore, TWE caused marked increase in urinary Na^+ and K^+ levels and reduction in pH of urine suggesting that it mainly acts as a loop diuretic. **Conclusion:** The TWE exhibits significant ($p<0.05$) diuretic activity in rats and it acts similar to that of a loop diuretic. In addition, the generated information of the present study provides data which will help the correct identification and authentication of this medicinal plant, which may help in preventing adulteration.

Key words: *Tragia involucrata*, diuretic, standardization, phytochemicals, Wistar rats

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since, time immemorial, man has been using whole plants, parts of plants and plant extracts as medicines in different formulations to treat various ailments. Even with the great advances in modern medicine still about 25% of modern drugs are directly or indirectly derived from higher plants[1]. Thus, it is evident that plants still play a major role in health care. One such medicinal plant that is being extensively studied at present is *Tragia involucrata* Linn., belonging to family Euphorbiaceae. It is commonly known as "Wel-kahambiliya" in Sinhala and "Indian stinging nettle" in English. *Tragia involucrata* is a perennial, hispid herb, with scattered and stinging hairs. The stem is elongated, slender and twines around a support[2]. The hair greatly irritates the skin causing itching, inflammation and oedema. *Tragia involucrata* occurs in India, Sri Lanka, Burma and China. In Sri Lanka, it is common in waste grounds in the low country Jaffna, Anuradhapura, Minneriya, Galle, Matara, etc.[2]. It is considered as a weed of cultivation and waste grounds[3].

Experimentally, this plant shows a wide range of therapeutical properties such as, antidiabetic and hypolipidaemic[4], diuretic[5], anticancer[6], antiinflammatory and analgesic[7], wound healing[8], psychopharmacological[9], antiepileptic[10], antihistamine[11], anthelmintic[12], nematicidal[13], larvicidal[14] and mosquito repellant[15] activities.

According to the traditional physicians in Sri Lanka, the whole plant of *T. involucrata* is widely used for loss of appetite, cough, asthma, fever, cardiac diseases and urinary disorders. Sri Lankan traditional system of medicine is well known for treating many types of renal diseases, such as dysuria, cystitis, renal calculi and hypertension[16]. Scientific investigations have been carried out for plants such as *Ruta graveolens* leaves hot water extract[17], *Mucuna pruriens* ethanolic extract of the aerial part[18], *Trianthema portulacastrum* crude extract[19] and for *Poria cocos*[20] for their diuretic activity.

Diuretic activity of *T. involucrata* root[5] was evaluated previously, using petroleum ether, chloroform and aqueous extracts. However, traditional and ayurvedic physicians in Sri Lanka, use the decoction of the whole plant as a remedy for urinary disorders. Therefore, the present study was carried out to scientifically validate the diuretic activity of the whole plant of *T. involucrata* using the hot water extract. Moreover, hot water extract was prepared according to the method described in Ayurveda Pharmacopoeia[21]. No such study has been carried out so far to evaluate the diuretic activity of *T. involucrata* using the traditional decoction as the extract. In addition, the whole plant of *T. involucrata* was standardized according to the WHO[22] guideline.

MATERIALS AND METHODS

Plant material: Whole plants of *T. involucrata* were collected from Uva, Southern and Western provinces of Sri Lanka at flowering stage during the period of March-June, 2015. The plant was authenticated by the Curator of National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka. A voucher specimen was deposited at the National Herbarium of Department of National Botanic Gardens, Peradeniya and at the Herbarium, Institute of Indigenous Medicine, University of Colombo, Rajagirya, Sri Lanka for future reference (1-Rajagiriya). The Bulk plant material was cleaned, washed, shade dried and ground into powder and stored in air tight polythene containers with proper labeling.

Standardization of *Tragia involucrata* whole plant: Physico-chemical parameters such as, total ash content, acid insoluble, water soluble ash content, extractable matter of water, methanol, ethanol, ethyl acetate and dicholoromethane were determined for the whole plant of *T. involucrata* dry powder according to the methods described in guidelines of World Health Organization[22].

Determination of total ash content: The powdered material (5 g) was accurately weighed and placed in a previously ignited and tared crucible. The material was ignited to a constant weight by gradually increasing the heat to 550°C for 5 h until the material became white indicating the absence of carbon. Residual ash was allowed to cool in a desiccator and weighed. The content of total ash in air dried material was calculated.

Determination of acid insoluble ash content: To the crucible containing the total ash, 25 mL of 2 M hydrochloric acid was added. The crucible was covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with hot water and the rinsed content was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the acid insoluble matter was transferred to the original crucible, dried and ignited to a constant weight. The residue was cooled in a desiccator and weighed. Acid insoluble ash was calculated and percentage was taken in relation to the air dried material.

Determination of water soluble ash content: Water (25 mL) was added to a crucible containing the total ash and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water insoluble matter

was collected on an ashless filter paper and washed with hot water. The filter paper containing the insoluble matter was transferred to the original crucible and ignited to a constant weight. Residue was cooled in a desiccator and weighed and water soluble ash was calculated.

Determination of extractable matter: Extractable matter was determined using water, methanol, ethanol, ethyl acetate and dichloromethane.

Extractable matter-hot condition: Accurately weighed 4.0 g of coarsely powdered air dried sample of *T. involucrata* was placed in a glass stoppered conical flask. Water (100 mL) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h. Then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of water. The flask was shaken well and filtered rapidly through a dry filter paper (90 mm diameter, Whatman®). After that, 25 mL of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105°C for 6 h, cooled in a dessicator and weighed. Finally, extractable matter was calculated.

The same procedure was carried out using methanol, ethanol, ethyl acetate and dichloromethane separately.

Extractable matter-cold condition: Accurately weighed 4.0 g of coarsely powdered air dried sample of *T. involucrata* was placed in a glass stoppered conical flask, macerated with 100 mL of water for 6 h, shaken frequently and then allowed to stand for 18 h. It was filtered rapidly with qualitative filter paper (90 mm diameter, Whatman®). After that, 25 mL of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Finally, dried at 105°C in an oven for 6 h, cooled in a desiccator for 30 min and weighed without delay. The content of extractable matter in water was calculated.

The same procedure was carried out using methanol, ethanol, ethyl acetate and dichloromethane separately.

Investigation of diuretic activity of *Tragia involucrata*: Diuretic activity of *T. involucrata* was investigated using the hot water extract of the plant.

Preparation of *Tragia involucrata* hot water extract (TWE): In brief, 60 g of the plant material was boiled in 1920 mL of distilled water (DW) and the final volume was reduced to 240 mL by gentle boiling over 4 h. The hot water extract was freeze dried and stored at 4°C until use (yield 10.8% dry weight basis).

Phytochemical screening: Qualitative screening of TWE for alkaloids, polyphenols, flavonoids, steroids, saponins and tannins was carried out according to Yadav and Agarwala[23].

Quantitative determination of total polyphenolic content: The total polyphenolic content was estimated according to the Folin-Ciocalteu method[24]. Known concentrations of TWE (0.1 mL) was diluted with distilled water (0.9 mL) and mixed with 5 mL of 10 fold diluted solution of Folin-Ciocalteu reagent. Four milliliters of saturated sodium carbonate solution was added to the above mixture and shaken. The absorbance of the reaction mixture was measured at λ 765 nm after 2 h. Total phenolic content was expressed as gallic acid equivalents (mg GAE g^{-1} extract).

Quantitative determination of total flavonoid content: The total flavonoid content was determined using the Dowd method[25]. In this experiment, 5 mL of 2% $AlCl_3$ in methanol was mixed with the same volume of TWE in known concentrations. After 10 min the absorbance of the reaction mixture was measured at λ 415 nm. Total flavonoid content was expressed as quercetin equivalents (mg qE g^{-1} extract).

Animals: Healthy adult male Wistar rats (weighing 200-225 g) were used throughout the experiment. They were housed in rat cages under standardized animal house conditions (room temperature: 25±3°C with 12 h dark/light cycles) and fed with standard rat feed and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. Ethical clearance for the animal studies was obtained from the Ethical Review Committee of Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka (Project No. RP/2013/12).

Administration of doses: Doses of TWE at 550, 1100, 1650 and 2200 mg kg^{-1} were administered orally by gastric gavage (each dose in a volume of 2 mL of DW) to separate groups of rats. The dose of TWE at 550 mg kg^{-1} corresponds to the normal therapeutic dose administered to adult humans as calculated on the basis of relative surface areas of humans and rats[26].

Evaluation of the diuretic activity: Thirty six Wistar rats were fasted for 18 h and divided into 6 groups (6 rats per group).

Their urinary bladders were emptied by gentle compression of the pelvic area and by pulling of their tails. One hour prior to the treatment, all animals received physiological saline (NaCl 0.9%) at an oral dose of 5 mL/100 g b.wt., to impose a uniform water and salt load[27].

Rats in groups 1-4 were treated orally with 550, 1100, 1650 and 2200 mg kg^{-1} of TWE in 2 mL of distilled water, respectively. Rats in groups 5 and 6 were treated orally with 2 mL of distilled water (control group) and the reference drug, 13 mg kg^{-1} furosemide, respectively. Each of these rats was individually placed in metabolic cages and urine output was determined at hourly intervals for 5 h. The colour of urine was also noted. In order to ascertain the mechanisms of action, the urine collected from groups 1-6 were subjected to the following investigations: pH (Consort C 533, multi-parameter analyzer), Na^+ and K^+ levels using atomic absorption spectrophotometry-flame photometry (Schimedzu, Japan) and specific gravity.

Statistical analysis: Results were expressed as mean+standard error of mean (SEM). Statistical analysis of the data was performed with one way analysis of variance (ANOVA) (IBM SPSS statistics 22). Significant differences were indicated by $p \geq 0.05$.

RESULTS AND DISCUSSION

Due to the widespread use of herbal medicine, standardization has become essential to obtain proper quality control profile for medicinal herbs used in traditional and indigenous medicine systems. Standardization of herbal powders helps to identify, authenticate the plant material, which are pre requisites of quality assurance of herbal drugs. This will ensure reproducibility which contributes to the safety and efficacy of the herbal drug.

The physico-chemical parameters of *T. involucrata* are illustrated in Table 1. The ash consists mainly of oxides of metals, salts and inorganic constituents which occur naturally, or may have adhered to the plant, or deliberately added to the plant as an adulteration. Thus, ash value is useful in estimating the purity of a crude plant material. In this study, the ash value was determined using three different methods, which are total ash, water soluble ash and acid insoluble ash. As mentioned in WHO guidelines[22] the total ash method is designed to measure the total amount of material remaining after the ignition which includes both "Physiological ash", derived from the plant tissue itself and "Non-physiological ash", the residue of the extraneous matter, such as sand and soil adhering to the plant surface. Acid insoluble ash indicates the presence of silica which shows the plant material being contaminated with earth or sand. Water soluble ash gives an idea about the water soluble salts present in the plant material. The ash values are important quantitative standards.

The extractive value is useful for the evaluation of crude plant material as it gives an idea about the nature of chemical constituents present in the plant material. It is also useful for the estimation of chemical constituents soluble in a specific solvent used for extraction. According to the results, extractive value of hot water extract is higher compared to cold water extract. It is also evident that water soluble extractive value proved to be higher than any other solvents. In indigenous preparations, *T. involucrata* is mostly given in the form of decoction in which the medium of extraction is water. There was a consistent reduction in extractive values observed with a decreasing order of solvent polarity (Table 1).

Screenings of phytochemicals are of great value since the bio-activity of plant materials are initiated by these phytochemicals. In the present study, some major phytochemicals were screened in the *T. involucrata* whole plant using hot water extract (Table 2). Gobalakrishnan *et al.*[28]

Table 1: Values related to physicochemical parameters of *Tragia involucrata* Linn., results are expressed as (Mean±SEM)

Total ash content	8.15±0.03%
Water soluble ash content	1.90±0.06%
Acid insoluble ash content	0.80±0.01%
Cold water extractive value	29.3%
Hot water extractive value	30.4%
Cold methanol extractive value	8.8%
Hot methanol extractive value	6.2%
Cold ethanol extractive value	3.6%
Hot ethanol extractive value	5.1%
Cold ethyl acetate extractive value	1.5%
Hot ethyl acetate extractive value	2.0%
Cold dichloromethane extractive value	1.6%
Hot dichloromethane extractive value	1.4%

Values are expressed as Mean±SEM

Table 2: Phytochemical screening of *Tragia involucrata* Linn., hot water extract

Phytochemical classes	
Alkaloids	-
Coumarins	+
Flavonoids	
• 10% lead acetate test	+++
• Dilute NH_3 and concentrated H_2SO_4	+
Glycosides	++
Cardiac glycosides	-
Keller-Kiliani test	
Steroid glycosides	-
Liebermann's test	
Saponins	++
Froth test	
Tannins	+++
Feric chloride test	
Terpenoids	++
Salkowski test	

-: Absence, +: Presence in low concentration, ++: Presence, +++: Presence in high concentration

have detected alkaloids in the fresh leaf extract of *T. involucrata.* Further, Dash *et al.*[29] have detected alkaloids in *T. involucrata* dry root extract of ethyl acetate extract and chloroform extract. However, in this study, alkaloids were not detected in the hot water extract. This may be due to the different geological conditions, changes in nutrition value of the soil, time of harvesting, etc. In addition, the whole plant was used in the present study unlike in the previous studies where only a certain part of the plant had been used. It might therefore be due to alkaloids being present at low quantity in the whole plant in relation to a high quantity in the leaf and dry roots.

High amounts of coumarins were present in the TWE. According to Venugopala *et al.*[30], coumarins are said to have multi biological activities, such as anti-inflammatory, anti-viral, anti-bacterial, anti-fungal, anti-cancer, anti-tumor, etc. Flavonoids are also present in high concentrations in TWE. It can be assumed that flavonoids present in *T. involucrata* are more soluble in high polar solvents than low polar solvents. Flavonoids are excellent anti-oxidants[31]. Hence, it can be presumed that the anti-oxidant activity of *T. involucrata* is greater in extracts of high polar solvents. Glycosides had not been detected previously in *T. involucrata* whole plant[32]. However, in the present study glycosides were detected in *T. involucrata*. Cardiac glycosides increase the contraction force of the heart[33]. Hence, it is used in the treatment of congestive heart failure. However, cardiac glycosides and steroidal glycosides were not detected in TWE. Saponins which protect the body against hypercholesterolaemia and function as an antibiotic[34] were present in TWE. The phenomenon of hemolysis is also common in plants containing saponins. Saponins cause hemoglobin to diffuse into the surrounding medium which is known as the haemolytic activity of the plant. Tannins have activities, such as anti-microbial, accelerate blood clotting, reduce blood pressure and reduce serum lipid levels[35]. Further, terpenoids which have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial[31] were present in TWE in high concentrations. The total polyphenolic content and total flavonoid content of TWE was 20.6±0.60 mg GAE g^{-1} extract and 15.8±0.30 mg qE g^{-1} extract, respectively.

Medicinal plants have become significant source of diuretics due to the increased side effects of modern diuretics. Although some of the complications related to modern diuretics may be mitigated with careful monitoring, dosage adjustment and replacement of electrolyte losses, some side effects are idiosyncratic and cannot be prevented[36].

An estimated 650 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 75 plants are in clinical use[37] as herbal diuretics. Investigators have demonstrated that herbal plants used as diuretics in traditional medicine have increased in recent years and might be beneficial as a tool in treating hypertension which is one of the dangerous complications of diabetes mellitus[38]. In the present study, diuretic effect of TWE was evaluated. The results showed that there is a significant ($p<0.05$) dose-dependent diuretic activity and natriuretic and kaleuretic activity in urine excretion (Table 3). Since, the diuretic activity of TWE is dose-dependent, it is evident that the effect is intrinsic and may not have been the result from nonspecific action.

After 5 h, a maximum diuresis (in terms of total urine output) was observed at a dose of 1650 mg kg^{-1} in healthy male Wistar rats. Urine excretion was gradually increased with the increased dose and at 1650 mg kg^{-1} it showed the maximum activity (Table 3). The same response was observed in 2200 mg kg^{-1}, thus, causing the dose response curve to become a bell shaped curve. Furthermore, urine output was measured in each group at hourly intervals up to 5 h. The urine output of all the rats treated with TWE was significantly ($p<0.05$) higher than that of the rats in the control group at both 1 and 2 h. Among the tested groups, the urine output of the rats treated with 1650 and 2200 mg kg^{-1} doses of TWE were significantly ($p<0.05$) higher than that of the rats treated with the reference drug, furosemide at 1 h. The maximum diuretic activity of all the tested groups were observed within 1 h (Fig. 1) and the activity was gradually

Table 3: Total urine volume, diuretic action, electrolyte levels, pH and specific gravity of urine in healthy Wistar rats treated with different doses of hot water extract of whole plant of *Tragia involucrata* L.

Group	Total urine volume	Diuretic action	Diuretic activity	Na^+ ppm	K^+ ppm	Natiuretic index Na^+/K^+	pH	Specific gravity
Control	1.30±0.28	-	-	1537.3±129.5	2461.7±51.2	0.62	6.3	1.0009
Furosemide	3.70±0.60*	2.85	-	4398.7±114*	2190.0±313.5*	2.01	5.7	1.0065
550 mg kg^{-1} of TWE	1.68±0.32*	1.29	0.45	4655.7±126.3*	6024.3±79.2*	0.77	5.9	1.0057
1100 mg kg^{-1} of TWE	1.93±0.18*	1.48	0.52	3189.3±99*	3910.0±122.3*	0.82	7.9	1.0003
1650 mg kg^{-1} of TWE	2.83±0.42*	2.17	0.76	6724.7±198.7*	5331.7±193.2*	0.26	6.4	1.0096
2200 mg kg^{-1} of TWE	2.80±0.05*	2.15	0.75	8425.0±78.1*	6580.3±133.0*	1.28	6.7	1.0106

Values are expressed as Mean±SEM, *Significant when compared with control, $p \leq 0.05$, n = 6

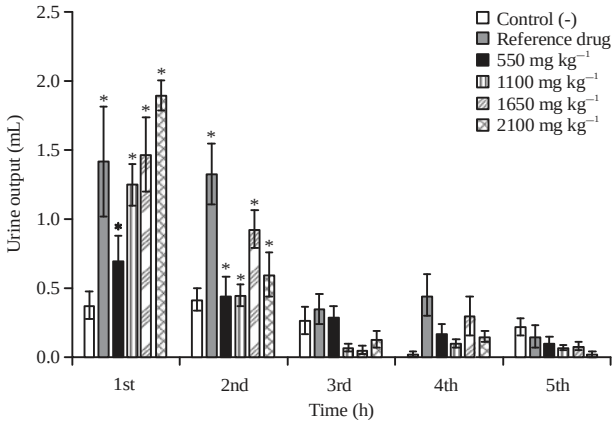

Fig. 1: Time course of diuresis in rats treated with different doses (550, 1100, 1650 and 2200 mg kg^{-1}) of hot-water extract *Tragia involucrata* L., whole plant, vehicle and reference drug, furosemide, values are expressed as Mean±SEM, *Significant when compared with control, $p \leq 0.05$, n = 6

declined with time. Compared to the urine output of rats treated with the reference drug furosemide, rats that treated with 1650 and 2200 mg kg^{-1} doses of TWE were significantly high.

According to Indian researches[5], diuretic activity of *T. involucrata* was evaluated for pet-ether, chloroform and aqueous extracts using the roots of the plant. Among the tested extracts, aqueous extract of *T. involucrata* showed the maximum diuretic activity with 2.61 diuretic action and 0.94 diuretic activity. In the present study, aqueous extract prepared from *T. involucrata* whole plant at a dose of 1650 mg kg^{-1} showed maximum diuretic activity with 2.17 of diuretic action and 0.76 of diuretic activity.

The TWE did not accompany with a reduction in urinary K^+ levels and there was no alkalization of urine. Therefore, it can be suggested that the TWE is not acting as a potassium-sparing diuretic[39]. Since, the TWE shows an increase in Na^+ excretion (Table 3) it can be ruled out that it is unlikely the extract acts as a thiazide diuretic, because thiazides increase the sodium reabsorption in the cortical collecting duct, which indirectly raises potassium excretion[40]. Furthermore, the diuresis of TWE was similar to that of furosemide accompanied with a marked increase in both urinary Na^+ and K^+ levels with slight acidification of the urine (Table 3), which are features of loop diuretics. Loop diuretics are powerful diuretics which enter the tubular lumen by secretion in the proximal convoluted tubule. These agents bind to the chloride-binding sites in the thick ascending loop of Henle, inhibiting its action which is responsible for a substantial proportion of sodium reabsorption in the nephron[41]. Their kaleuretic effect results both from the inhibition of K^+ reabsorption along the thick ascending limb of Henle's loop and from increased Na^+ delivery to the distal tubule[41]. Therefore, these diuretics causes natriuresis and kaleuresis. Further, these diuretics also cause acidification of urine[39]. Therefore, it can be suggested that TWE may be acting as a loop diuretic.

Phytochemical screening of whole plant of TWE, showed the presence of active phytochemical groups such as flavonoids and saponins in high concentrations (Table 2). Previous studies suggests that these phytochemical groups are responsible for diuretic activity by affecting the physiological processes of the renal system, such as local irritation of kidney epithelia[42], increase potassium sparing capacity[43], adenosine A1 receptor binding activity[44], elevation of glomerular filtration rate[45], increasing renal vaso-relaxation thereby increasing kidney filtration[46]. Therefore, a possibility exists that one of the mechanisms of diuretic activity may be due to the result of the presence of these phytochemicals.

Loop diuretics are clinically used in pulmonary oedema, chronic heart failure and hypertension[47]. Since the diuresis action of TWE is similar to that of loop diuretics the extract may be useful as a natural therapeutic agent in the treatment of above conditions. Most loop diuretics such as furosemide and bumetanide are organic ions. As mentioned earlier loop diuretics enter the tubular lumen by secretion in the proximal convoluted tubule and secretion may be impaired in the presence of accumulated organic anions which occurs in chronic renal failure, chronic liver failure and also drugs, such as cimetidine and trimethoprim compete for this secretion pathway[40]. In such cases herbal diuretics, such as TWE might be beneficial. One major limitation of this extracts is that the increased risk of hypokalaemia similar to other loop diuretics. If the above results are applicable to humans, it will be an important finding clinically both locally and globally since 80% of people in developing countries still rely on traditional medicine[48]. Further phytochemical, pharmacodynamic investigations should be carried out to find the exact mechanism of diuretic effect and the long term use of TWE as a diuretic.

Safety of a herbal extract is just as important as its efficacy. Even if an extract is most efficacious, in terms of its activity, it will still be no use if the extract exhibits any toxic effect. Moreover, toxicity studies are imperative especially when high doses are involved. A sub-acute toxicity study was performed for TWE. The extract did not produce any signs of hepatotoxicity (in terms of AST, ALT and ALP) or renotoxicity (in terms of creatinine and urea) or unacceptable

hematological effects in terms of RBC, WBC, differential WBC, %PCV, MCV, MCH, MCHC and Hb concentration at an upper limit dose of 5000 mg kg^{-1} given for 14 consecutive days. Therefore, TWE can be safely used as a diuretic at a dose of 1650 mg kg^{-1} since this dose showed the maximum urine output.

CONCLUSION

In the present study, diuretic activity of *T. involucrata* was scientifically investigated for the first time using the aqueous extract of the whole plant. *Tragia involucrata* exhibits significant diuretic activity and it acts similar to that of a loop diuretic. In addition, the generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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