Research Article

Evaluation of diuretic potential of petroleum ether extract of *Dendrophthoe falcata* leaves in wistar rats

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Abstract

Objective: The present study was undertaken to establish the diuretic activity of petroleum ether extract of leaves of *Dendrophthoe falcata* (EEDF) in wistar rats. **Materials and Methods:** The Petroleum ether extract of leaves of *Dendrophthoe falcata* were administered to experimental rats orally at doses of 250mg/kg and 500mg/kg and compared with furosemide (20mg/kg., p.o) as the standard. The parameters measured for diuretic activity were total urine volume, urine concentration electrolytes such as sodium, potassium, and chloride. **Results and Conclusion:** The rats treated with EEDF at doses 250mg/kg and 500mg/kg showed higher urinary output when compared to respective control and also showed dose dependent increase in the excretion of electrolytes when compared to the control group. The extract has diuretic effect supporting the ethno-pharmacological use as diuretics. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

Keywords: *Dendrophthoe falcata*, diuretic action, diuretic index, lipschitz value

Introduction

Diuretics that enhance the rate of urine flow and sodium excretion are used to maintain the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is helpful in many life-threatening conditions such as congestive cardiac failure (CCF), nephritic syndrome, cirrhosis, renal failure, toxemia of pregnancy, premenstrual tension, and hypertension (Pandya et al., 2012; Sravani et al., 2010). The presently available diuretics such as thiazides and loop diuretics exhibit several adverse effects like electrolyte imbalance and metabolic alterations (Hullatti et al., 2011) etc.

Today medicinal plants are very important for the growth of new drugs. People are using herbal drug because of its safety, efficacy and lesser side effects. Plants and plant products have utilized with varying success to cure and prevent diseases. At present demand of natural plants derived products are increasing day by day in global countries. The significance of medicinal plants in national economy and its potential for the rapid growth of herbal products have been emphasizing frequently (Ankur et al., 2010).

The plant kingdom plays an important role in the life of humans and animals. India is the largest producer of medicinal plants and is rightly called as "Botanical garden of the world". Medicinal plants have stated to compromise about 8000 species and account for approximately about 50% of all higher flowering plant species of India. In other words there are about 400 families of the flowering plants; at least 315 are represented by India. In recent years, the use of traditional medicine information on plant research has again received considerable interest. The western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources have become acknowledged by most academic and industrial researchers (Pandey and Madhuri, 2010).

Dendrophthoe falcata is also known as "Vanda" in the Indian Ayurvedic system of Medicine. *Dendrophthoe falcata* (L.f) Ettingish is a perinneal climbing woody parasitic plant. It is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar It is widely distributed throughout India. The whole plant is used in indigenous system of medicine as cooling, bitter, astringent, aphrodisiac, narcotic, and diuretic

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and is useful in treating pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesical calculi and vitiated conditions of kapha and pitta. Also decoction of plant is used by women as an antifertility agent, also have anticancer activity. Leaf paste is used in skin diseases. Its paste is applied on boils, setting dislocated bones and extracting pus (Nadkarni K M 2000; Pattanayak and MitraMazumder 2009; Sarvanimanthri et al., 2011). Hence, the present study is done to evaluate the diuretic potential of petroleum ether extract of *Dendrophthoe falcata* leaves in wistar rats.

Materials and methods

Collection of plant material and authentication

Leaves of *Dendrophthoe falcata* (Indian Mistletoe) were collected in and around the areas of Cherlapally, Medchal Dist, Telangana, India.

Extraction of plant material

The leaves were washed with water to remove extragenous matter; and were shade dried for 15days. This was dried at room temperature under shadow conditions. The dried leaves were homogenized to get coarse powder and were stored in air tight container until extraction. The powdered material (100 g) was subjected to continue hot extraction in soxhlet apparatus at a temperature of (60-70 ^{0}C) by using ethanol (95% v/v) as solvent. After complete extraction, the extract was dried (Chinnala et al., 2013; Crossland, 1980).

Experimental animals

Male albino Wistar rats weighing between 180-220gms were used for the study. The animals were fed commercial rats feed pellets (Hindustan Lever Ltd., Bombay, India) and were given water *ad libitum*. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments in animal house approved by CPCSEA (1832/PO/RE/S/15/CPCSEA). They were housed in polypropylene cages under proper humidity 55±5% and temperature: 25±2^{o}C condition and maintained on normal 12-12h day-night cycle. The experimental protocol and all procedures were approved by Institutional Animal Ethical Committee (IAEC 003/IAEC/NNRG/2017).

Evaluation of Diuretic activity

Male albino Wistar rats weighing between 150-200 gm were taken and used for this experiment. The animals were grouped into four groups, each consisting of six animals and they were fasted and deprived of food and water for 18 hours prior to the experiment. The first group was treated as normal group which received only 0.9% NaCl solution 25ml/kg/ p.o. The second group served as the standard group, received the standard drug Furosemide 20mg/kg/p.o. The third and fourth groups received petroleum ether extract of *Dendrophthoe falcata* (EEDF) of 250mg/kg/p.o and 500mg/kg/p.o. suspended in 0.9% NaCl Solution.

Assessment of diuretic activity

Collection and analysis of urine

After Oral administration each animal was placed in an individual metabolic cage specially designed to separate feces and urine at room temperature. The Observed parameters were total urine volume for 5 hours, Na^+, K^+, Cl^- excreted in urine.

The concentration of the electrolytes in urine is expressed in terms of mmol/l and the urine volume is expressed in ml/100g/5 hours. Na^+, K^+ concentrations were measured by Flame Photometer and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate as an indicator.

Computation of diuretic parameters

Diuretic parameters were determined as in Equations 1-2 (Danamma et al., 2011; Abdala et al., 2012).

Diuretic index = Vt/Vc…… (1)

Where Vt is mean urine volume of test group and Vc is mean urine volume of control group.

Lipschitz value = Vt/Vr…….. (2)

Where Vt is mean urine volume of test group and Vr is mean urine volume of reference group.

Saluretic index = Ct/Cc…….. (3)

Where Ct is the concentration of electrolyte in urine of test group and Cc is the concentration of electrolyte in urine of control group (Muhammad Asif et al., 2013).

Statistical analysis

The results were expressed as mean ±SD among data were determined using ANOVA followed by Dunnett's test as per suitability $P<0.05$ was considered as significant, $p<0.01$ was considered as very significant, $P<0.001$ was considered as highly significant.

Results and discussion

The yield obtained from petroleum ether extract of *Dendrophthoe falcata* was about 16.2g (1.06%).

Phytochemical analysis

In the preliminary phytochemical studies of leaf extract of *Dendrophthoe falcata* confirmed the strong presence of desired phytochemicals in petroleum ether extract. Hence, for the further studies petroleum ether extract of *Dendrophthoe falcata* have been selected.

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of

Table 1. Diuretic activity of petroleum ether leaf extract of *Dendrophthoe falcata*

Groups	Urine Volume (ml/100g/5h)	Urine pH	Urinary Excretion (mmol/l)			Diuretic Index	Lipschitz value	Saluretic Index	
			Na^+	K^+	Cl^-			Na^+	K^+
Group I	4.15±0.10	6.03±0.06	67.6±1.75	69.33±1.03	56.67±1.50	--	--	--	--
Group II	8.95±0.48	6.71±0.005	143.3±2.16	162±1.41	93.5±1.64	2.15	--	2.11	2.33
Group III	5.33±0.13**	6.67±0.005**	93.5±1.04**	83±0.89**	63.5±1.049**	1.28	0.59	1.38	1.19
Group IV	5.76±0.34**	6.69±0.005**	112.8±3.54**	123.2±1.94**	76.33±0.81**	1.38	0.64	1.66	1.77

Each value is expressed as the mean ±SD (n=3) one-way ANOVA, **p<0.001 when compared with the normal group

volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus, diuretics play an important role in hypertensive patients (Patel et al., 2009). The preliminary phytochemical investigation revealed the presence of flavanoids and tannins in the EEDF. Studies have established that there are numerous compounds which could be accountable for the plant's diuretic effects such as (eg., flavanoids, saponins or organic acids) (Dubeyet al., 2010). Studies have shown that diuretic activity of flavanoids may be due to their binding with adenosine A^1 receptors (Yulianaet al., 2009).

Urine volume (ml), urine pH, concentration of electrolytes in urine such as sodium, potassium and chloride were the parameters measured while assessing the diuretic potential of the extract. The diuretic index of 5 hour urine samples was calculated to assess the diuretic potential of ether extract of *Dendrophthoe falcata* leaves. The present study revealed that, ether extract of *Dendrophthoe falcata* significantly increased ($p<0.001$) the urinary output at a dose of 250 mg/kg and 500mg/kg when compared to normal group (Table 1) (Figure.1). When compared with group I, approximately increase in urine output was observed in test groups, respectively. The diuretic index values of test groups (group III and IV) were 1.28 and 1.38, respectively indicating a good diuretic activity while maximum diuretic effect was observed at the dose of 500 mg/kg of extract (Table 1). The Lipschitz values showed that at the doses of 250 mg/kg and 500 mg/Kg of petroleum ether extract of *Dendrophthoe falcata* leaves was having 59 % and

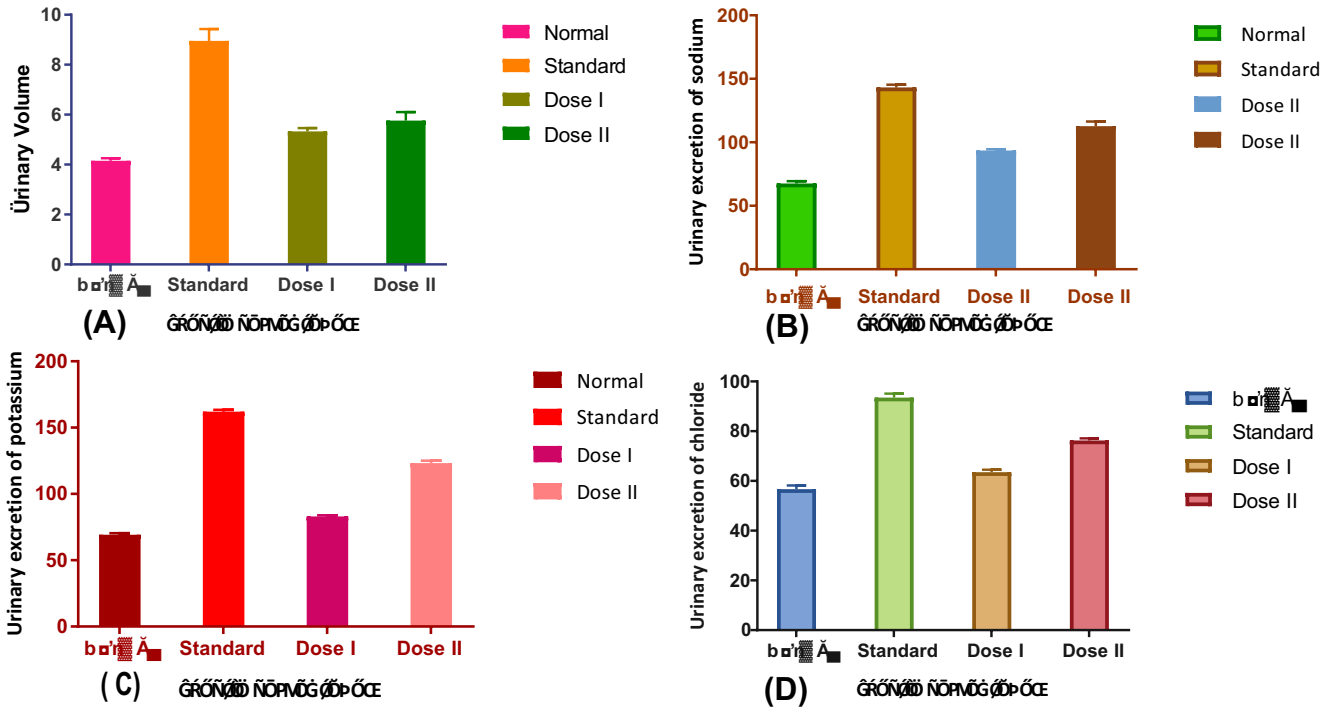

Figure 1. Effect of *Dendrophthoe falcata* extract on different parameters of diuretic activity: (A) 5hr Urinary volume; (B) 5hr sodium concentration in urine; (C) 5hr potassium concentration in urine; (D) 5hr chloride concentration in urine

69 % of diuretic activity, respectively compared with furosemide (Table 1). Present study shows that the ether extract of *Dendrophthoe falcata* leaves possess good diuretic activity. Increased water intake and increased urinary output decrease the incidence of urinary calculi in those patients who are predisposed to the disease (Srinivas et al., 2012). The Na^+ ion concentration of standard drug was 143.3±2.16, the EEDF 250mg/kg treatment shown 93.5±1.04 and the EEDF 500mg/kg treatment shown 112.8±3.54 (Figure.2), K^+ ion concentration of standard drug was 162±1.41, the EEDF 250mg/kg treatment shown 83±0.89 and the EEDF 500mg/kg treatment shown 123.2±1.94 (Figure.3) and Cl^- ion concentration of standard drug was 93.5±1.64, the EEDF 250mg/kg treatment shown 63.5±1.049 and the EEDF 500mg/kg treatment shown 76.33±0.81(Figure. 4). Na^+ ion, K^+ ion and Cl^- ion concentrations significantly increased ($p<0.001$) when compared to normal group. The Saluretic index values of sodium levels of test groups (III and IV) were 1.38 and 1.66 and potassium levels of test groups (III and IV) were 1.19 and 1.17 (Table 1).

In primary hypertension, sodium is considered an important external factor. Numerous studies have shown the adverse effects of increased sodium uptake on arterial blood pressure (Horacio et al., 2007). Increased excretion of urinary sodium in our experimental animals after the administration of EEDF showed that plant is a potential candidate to be used as antihypertensive agent (Cheung et al., 2000).

Conclusion

The goal of pharmacological studies on medicinal plants should not be restricted to find new prototype pure compounds as drugs. Active extracts, fractions or mixture of fractions/extracts may prove very effective drugs. Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials. The manufacture of plant products should be governed by standards of safety and efficacy.

The presented data about various parameters like Urinary volume, urine pH, electrolyte concentrations (Na^+, K^+ and Cl^-) indicates that the administration of ether extract of *Dendrophthoe falcata* leaves has shown the diuretic potential by increasing the urine formation, supporting folklore information regarding diuretic activity of the plant; it is also used for the treatment of hypertension and renal disease. The extract has diuretic effect supporting the ethno-pharmacological use as diuretics. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

Conflicts of interest: Not declared.

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