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Evaluation of Diuretic Activity of Ethanolic Extract of *Ocimum Sanctum* (L) in Wistar Albino Rats

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ABSTRACT

Ocimum sanctum L., a popular herbal remedy with a myriad of medicinal properties has been well studied. Leaves and seeds of this plant have been reported to reduce blood and urinary uric acid level and claimed to have diuretic property. This study attempts to explore the diuretic activity of aqueous extract of *Ocimum sanctum* in healthy Wistar albino rats.The study was conducted in saline primed Wistar albino rats (n=6) using frusemide (20 mg/kg per oral) as the reference diuretic drug with two oral doses of ethanolic extract of *Ocimum sanctum* (L.) 250mg/kg and 500mg/kg respectively. Urine volume and electrolytes (Sodium, Potassium and Chloride) excretion was estimated at the end of 24 hours. Data was analyzed by ANOVA followed by Tukey's test. $P < 0.05$ was considered as statistically significant. Ocimum sanctum extract significantly increased the volume of urine (5.48±0.13ml/100g/24hr and 7.52±0.19ml/100gm/24hr), increasing the diuretic index to 1.65 and 2.26 for 250mgkg and 500mg/kg dose ranges respectively ($P< 0.01$). The test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride excretion. There was an increase in the saluretic index as reflected by the Na/K ratio to 2.2 and 2 respectively for the two dosages studied when compared to frusemide which showed a saluretic index of 1.81. These findings support the use of *Ocimum sanctum* as a diuretic agent with an action similar to that of the loop diuretic, frusemide. Further studies with larger doses and longer duration exploring the exact mechanism of action are warranted.

Keywords: Diuretic, *Ocimum sanctum*, saluretic, frusemide, Lipschitz method

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INTRODUCTION

Diuretics, either alone or in combination with other drugs, are valuable in the treatment of hypertension, congestive heart failure, ascites & pulmonary edema [1-5]. Two widely used diuretics, thiazides and the high ceiling loop diuretic, furosemide, have been associated with a number of adverse effects such as electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the rennin angiotensin neuro endocrine systems and impairment of sexual function [1,4]. Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but lack scientific authentication. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [6, 7].

Ocimum sanctum Linn. (Family: Labiatae), locally known as 'Tulsi' in Hindi and 'Holy Basil' in English, is an erect soft hairy aromatic herb or under shrub found throughout India. It has a high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine. In Ayurveda, Tulsi *(Ocimum sanctum L.)* has been well documented for its therapeutic potential and described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) [8]. Several medicinal properties have beenattributed to *Ocimum sanctum* L. Different parts of Tulsi plant e.g. leaves,flowers, stem, root, seeds etc. are known topossess therapeutic potentials and have been used by traditional medical practitioners, as expectorant, analgesic, anticancer, anti-asthmatic, antiemetic, diaphoretic, anti-diabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic and anti-stress agents. Tulsi has also beenused in treatment of fever, bronchitis, arthritis, convulsions etc. [8].

Various experimental and clinical studies have reported the anticancer, chemo-preventive, antioxidant, antimicrobial, radio-protective antihypertensive and cardio-protective activities of *Ocimum sanctum*[9].Various other properties reported include analgesic, anti-inflammatory, antipyretic, memory enhancer, hepatoprotective, antifertility, anti-diabetic, anti-arthritic, antiulcer, adaptogenic and anti-stress, anti-cataract, anticoagulant and central nervous system depressant activities. Acute and sub-acute toxicity studies have also proven its excellent safety profile [8-10].

Though traditional systems of medicine have claimed that *O.sanctum* can be used as a diuretic agent, literature search revealed very few studies exploring the diuretic potential of this plant. The present study was undertaken to explore and authenticate the diuretic potential of alcoholic extract of the leaves of *Ocimum sanctum* in Wistar albino rats.

MATERIALS AND METHODS

Experimental animal

Adult male Wistar albino rats (150-200 g) from our breeding stock were used for the study. They were housed in clean and transparent poly propylene cages with three animals in

each cage and maintained at 27℃ with 12: 12 h light-dark cycle for a period of 7 days prior to the study. They were fed standard rat chow and water *ad libitum*. The experimental procedures described were approved by the Institutional Animal Ethics Committee.

Drugs

Frusemide (Sanofi Aventis Co.) was used as a reference diuretic drug. The test drug extract of *Ocimum sanctum* (70% alcohol extract of leaves), was obtained from Natural Remedies Pvt. Ltd, Bangalore.

Evaluation of diuretic activity

Each animal was placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. The method of Lipschitz*et al*, [11,12] was employed for the assessment of diuretic activity. According to this method, the animals, deprived of food and water for 18 hours prior to the experiment, were divided into 5 groups (n=6). Group I animals received normal saline (25 ml/kg, p.o.); Group II received the standard diuretic Frusemide (20mg/kg body weight, p.o.) and Groups III and IV received the test compound, alcoholic extract of leaves of *Ocimum sanctum*(250mg/kg and 500mg/kg body weight p.o.) respectively. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5ml/100g body weight to impose uniform water and salt load [13].All the drugs were freshly prepared prior to administration.

Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. At the end of 5 h, the volume of urine collected was measured. During this period, no food and water was made available to animals. The parameters noted were body weight before and after test period, total urine volume, and concentration of Na^+, K^+ and Cl^- in the urine. Na^+, K^+, Cl^- concentrations were determined by Ion Sensitive Electrode; Roche Hitachi 917automatic analyzer and bicarbonate ion was estimated with Blood gas analyzer: AVLcompact-3.

Statistical Analysis

The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Effect on urine volume

There was no evidence of dehydration and the animals were found normal at the observed 5hr and 24hr intervals. The reference diuretic frusemide, significantly increased the urine output when compared to control ($P < 0.01$), the diuretic index being 2.74. The test drug

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at 250 and 500 mg/kg doses, showed a statistically significant increase in the diuretic index (1.65 and 2.26 respectively) when compared to the control (P<0.01) as shown in table no1.

Effect on urinary electrolyte excretion:

As indicated in table no 2, the test drug, when compared to the control group, showed a significant increase in the excretion of sodium and potassium excretion in a dose dependent manner (P <0.01).The increase in sodium and potassium excretion when compared to the standard frusemide was statistically insignificant although the saluretic index at 250mg/kg (2.19) and 500mg/kg (2.0) was greater than that of frusemide (1.81).

Table 1: Effect of oral administration of *Ocimum sanctum* leaf extract on urinary volume excretion

Group	Urine volume (ml/100g/24hr)	Diuretic index (24hr interval)†
Control	3.33 ± 0.13	-
Frusemide	9.12±0.25*	2.74
Ocimum Extract (250mg/kg)	5.48±0.13*	1.65
Ocimum Extract (500mg/kg)	7.52±0.19*	2.26

Values are expressed in mean ± SEM; * P <0.001 compared with control group (ANOVA followed by Tukey's test)
† Diuretic index = volume of test group/volume of control group

Table 2: Effect of oral administration of *Ocimum sanctum* leaf extracton urinary electrolyte excretion

Groups	Na^+	K^+	Saluretic index‡ Na/K		
			Na	K	
Control	107±2.1134	55±10.1			1.95
Frusemide	167.8±7.00*	92.5±2.06*	1.57	1.68	1.81
Ocimum Extract (250mg/kg)	144±4.97*	65.67±3.7*	1.35	1.19	2.19
Ocimum Extract (500mg/kg)	157.9±6.47*	78.93±4.21*	1.48	1.44	2.0

Values are expressed in mean ± SEM; * P <0.01 compared with control group (Kruskall Wallis and Mann Whitney test) ‡ Saluretic index = electrolyte concentration of test group/electrolyte concentration of control group

DISCUSSION

The present study revealed that the alcoholic extract of the leaves of *Ocimum sanctum* showed significant increase in urinary output and urinary electrolyte concentration comparable to frusemide. Diuresis has two components: increase in urine (water secretion) and a net loss ofsolutes (i.e. electrolytes) in the urine [14].These processes result from suppression of renal tubular re-absorption of water and electrolytes into the blood stream. The reference drug frusemide, increases urine output and urinary excretion of sodium by inhibiting Na^+ K^+2Cl^- symporter (co-transport system) in the thick ascending loop of Henle [14].

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles [15]. The regulation of Na^+/K^+ balance is also intimately related to renal control of acid-base balance. The K^+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [16].

In the present study, frusemide showed strong diuresis accompanied with high natriuresis, chloruresis, and kaliuresis (P<0.01).Further there was low Na^+/ K^+ ratio, as it inhibits Na^+ K^+ and Cl^- co-transport at the thick ascending loop of Henle. K^+ excretion was increased perhaps due to high Na^+ load reaching the distal tube. However, *Ocimum sanctum* leaves extract induced both marked natriuresis and kaliuresis ($p < 0.01$), but the Na^+/ K^+ ratio was more than that of frusemide, indicating the weak kaliuresis or K^+ saving property of the extract [17]. The above results raise the possibility of existence of diuretic activity by inhibiting tubular re-absorption of water and sodium ion. It is a good indicator for the efficacy of *O.sanctum* leaves extract as diuretics. A previous study evaluating the effect of leaves as well as seeds of *O. sanctum* on blood/urinary uric acid and urea levels while investigating its effect in gout has also reported that it produced diuresis but however no data on its saluretic activities were noted [18].

Phytochemical analyses have revealed that the leaves of *O. sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyleugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. Fresh leaves and stem of *O.sanctum* extract yielded some phenolic compounds (antioxidants) such ascirsilineol, circimaritin, isothymusin, apigenin androsameric acid, and appreciable quantities of eugenol. Two flavonoids, viz., orientinandviceninfrom aqueous leaf extract of *OS* have been isolated. Ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O glucuronide, orientinand molludistin have also been isolated from the leaf extract [19].

The active constituent producing diuretic activity could be attributed to the presence of ursolic acid, one of the major constituents of the Tulsi leaves, which has been suggested to possess diuretic activity [19]. Moreover, eugenol to which most of the medicinal activities of *O.sanctum* has been attributed could also be implicated as Boulos (1993) has reported that the buds of clove *Syzygiumaromaticum* and Bay leaf extracts *Laurusnobilis* which has eugenol as its main chemical constituent were used in folkmedicine as diuretic [20].

CONCLUSION

In conclusion, alcoholic extract of *Ocimum sanctum* leaves showed good diuretic activity in the experimental model studied. Further studies can be done using different models to evaluate the mechanism of action and compare with other known diuretics.

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