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Study on diuretic activity and electrolytes excretion of methanol extract of *Lippia nodiflora* (Verbenaceae) in rats

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SUMMARY

In the Indian traditional medicine, *Lippia nodiflora* (Verbenaceae) whole plant is claimed to possess powerful diuretic activity. However, the diuretic potential of this plant is not yet investigated. The aim of this study was to evaluate the diuretic potential of methanol extract of *Lippia nodiflora* (MELN) in rats. Control (0.9% saline solution, 25 ml/kg, b.w) or urea (1 g/kg b.w) or frusemide (5 mg/kg b.w) and different concentrations of MELN (200 and 400 mg/kg b.w) were intraperitoneally administered (n = 6 per each treatment group) to hydrated rats and their urine output was monitored over a period of 5 h and 24 h after drug administration. The diuretic responses with its electrolyte excretion potency of the extract were highly remarkable in comparison with control animals. The extract at doses of 200 and 400 mg/kg shows a significant increase in volume of urine with increase in Na^+, Ca^{2+} and Cl^- excretion accompanied by the excretion of K^+ in dose dependent manner. This study suggests that the active component(s) in MELN had similar diuretic effect to that of frusemide. These results validate the traditional use of *Lippia nodiflora* as a diuretic agent.

Keywords: *Lippia nodiflora*; Methanol extract; Urinary volume; Diuretic activity; Electrolyte excretion.

INTRODUCTION

Diuretics play an important role in situations of fluid overload, like acute and chronic renal failure, hypercalciurea, cirrhosis of liver and also as an antihypertensive agent. A number of diuretics like mannitol, thiazides, frusemide, and ethacrinic acid are used in practice. Still there is a need for more effective and less toxic diuretic. Many indigenous drugs have been claimed to have diuretic effect in Ayurveda system of medicine but they were not properly investigated. Among the several plants *Dolichus biflorus, Tribulus terrestris, Dendrophthoe falcate, Boerhaavia diffusa, Saccharum oficinarum, Butea frondosa, Boerhaavia repens, Boerhaavia rependa, Homonia riparia* have shown excellent diuretic activity (Harvey, 1966; Singh and Udupa, 1972; Rani, 1988; Srivastava *et al*., 1988; Ramachandra, 1989; Singh *et al*., 1991; Alekutty *et al*., 1993; Zafar, 1994).

Lippia nodiflora Mich (Verbenaceae) is a creeping perennial herb with small white flowers, a weed of wet ground and grassy pastures (Gamble, 1957; Chopra *et al*., 1958). The herb is known as poduthalai in Tamil. The plant is distributed throughout India, Ceylon, Baluchistan and Africa.

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The plant is diuretic, aphrodisiac, useful in diseases of heart, good for ulcers and bronchitis, useful in fevers and colds (Kirthikar and Basu, 1975). The herb possesses cooling, diuretic and stoppage of pain knee joints (Wealth of India, 1962; The Useful Plants of India, 1986). The plant made into a poultice used as maturant for boils (Nadkarni, 1954; Chopra *et al*., 1956). Aqueous extract of leaves of the plant were reported for the anti-inflammatory, analgesic and antipyretic activity in rodents (Caceres *et al*., 1991; Forestieri *et al*., 1996) and used for the treatment of gonorrhea (Zamora-Martinez and Nieto de Pascual, 1992). Antimicrobial activity was reported from the herb (Mukherjee, 1991).

There is paucity of data about the pharmacological activities of *Lippia nodiflora,* which prompted us to pursue this pharmacological evaluation of *Lippia nodiflora* whole plant to verify the medicinal properties. Therefore, the present study was undertaken to evaluate the diuretic activity of methanol extract of *Lippia nodiflora* in normal rats.

MATERIAL AND METHODS

Plant material and extraction

Lippia nodiflora was collected from Mallasamudram, Namakkal District, Tamilnadu, India. The plant material was taxonomically identified by the H.O.D, Department of Botany, Kuvempu First Grade College, Channapatna, Karnataka, India. A voucher specimen (No DAKJU-04/2005) has been preserved in our laboratory. The whole plant was dried under shade and then powdered with a mechanical grinder and stored in an air tight container, The dried powdered material was defatted with petroleum ether (60 - 80°C) followed by the extraction with methanol in a soxhlet apparatus. Phytochemical screening of the extract revealed the presence of flavonoids, saponins, triterpenes, phenolic compounds (tannins) and steroids (Kokate, 1997). Methanol extract of Lippia nodiflora (MELN) was dissolved in distilled water prior to administration for the pharmacological studies.

Animals

Male Wister albino rats (150 - 180 g) were purchased from Indian Institute of Chemical Biology, Kolkata, India. After screening, the animals were taken and kept in identical condition (12 h light: 12 h dark cycle) at 25 ± 0.5°C in the animal unit, Department of Pharmaceutical Technology, Jadavpur University, Kolkata atleast 7 days prior to pharmacological studies, with free access to pellet diet (Hindustan Lever Limited, Mumbai) and water *ad libitum*.

Chemicals and drugs

Petroleum ether (60 - 80°C) from E. Merck Limited, Mumbai, methanol and urea from Sisco Research Laboratories Pvt Ltd, Mumbai, frusemide (Lasix) was obtained from Aventis pharma limited, Thane. All other chemicals used were of reagent grade.

Acute toxicity study

Acute toxicity study was performed as per OECD-423 guidelines (acute toxic classic method), (Ecobichon, 1997). Wister rats of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only with water. The extracts were administered orally at the dose level of 5 mg/kg body weight and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose assigned as a toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100, 200, 400, 800, 1,600 and 2,000 mg/kg body weight. On the basis of LD_{50}, two doses were selected for detailed study.

Screening of diuretic activity

The method of Lipschitz *et al.* (1943) was employed for the assessment of diuretic activity, male albino rats weighing 150 - 180 g were selected, and the tail base was pressed to empty the bladder of remaining urine. The test animals were divided into five groups, containing six rats in each group. All the animals received normal saline (25 ml/kg, b.w) orally prior to start of the experiment. Group I

served as a control and was fed only with normal saline. Group II received urea (1 g/kg) as positive control and frusemide (5 mg/kg) as reference standard diuretic. Group IV and V received the test materials, (MELN) at doses of 200 and 400 mg/kg, b.w respectively. Immediately after dosing the rats were placed in metabolic cages and kept at room temperature of 25 ± 0.5°C. During this period, no food and water was made available to them. The urinary output of each group was recorded at 5th h and 24th h from the graduated urine chamber of metabolic cage. Animals were taken out of the cages and urine samples, which are collected from metabolic cage, were analysed for Na^+, K^+, Ca^{2+} and Cl^- in mMol/l (Mukherjee *et al*., 1996; Murugesan *et al*., 2000; Mamun *et al*., 2003). Urine samples were analysed for Na^+, $K^+$and Ca^{2+} concentration by a flame photometer (Chemito 1,020) while Cl^- concentration was determined titrimetrically (Indian pharmacopoeia, 1996). The pH values were measured with a pH meter (Mettler Toledo, Seven Easy) (Sheth *et al*., 1972). The instrument was calibrated with standard solutions containing different concentration of Na^+, K^+ and Ca^{2+} (Muneer *et al*., 2003).

The volume of urine excreted at 5 h and 24 h in each group of animals has been expressed as percent of liquid administered (Gujral *et al*., 1955). This percentage gives a measure of "urinary excretion" (U.E) - independent of group weight, thus

$$\text{Urinary excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100$$

The ratio, urinary excretion in test group: urinary excretion in control group has been used for the measure of diuretic action for the treated groups.

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test group}}{\text{Urinary excretion in control group}}$$

The relative diuretic potency can be determined by Van Armar (1954). To obtain the diuretic activity, the test groups (MELN) were compared with reference standard diuretic, Urea.

$$\text{Diuretic activity} = \frac{\text{Diuretic action of extract}}{\text{Diuretic action of Urea}}$$

The sum of Na^+ and Cl^- excretion was estimated for saliuretic activity. The ratio Na^+/K^+ was estimated as a natriuretic activity. The ratio $Cl^- / Na^+ + K^+$ (ion quotient) was derived to estimate carbonic anhydrase inhibition (Somova *et al*., 2003).

Statistical analysis

Results are mean ± S.E.M. Statistical analysis of control and test data was determined by ANOVA (SPSS computer software) followed by one-way analyses of variance were used for different doses within a group. *P* value of 0.001 was considered statistically significant.

RESULTS

The hot extraction of coarse powder (350 g) of *Lippia nodiflora* was carried out with petroleum ether and methanol, which yielded 2.91% and 21.42%, respectively. The phytochemical analysis showed the presence of flavonoids, triterpenes, phenolic compounds (tannins) and steroids.

Acute toxicity study

MELN did not cause any mortality up to 2,000 mg/kg and were considered as safe (X-unclassified) (OECD, 1996).

Effects on urine volume and diuretic activity

Table 1 and 2, showed that, the cumulative urine volume was measured at 5th h and again after 24th h of control (0.66 ± 0.006 and 2.24 ± 0.006), urea (0.83 ± 0.02 and 2.50 ± 0.01), frusemide (2.13 ± 0.01 and 4.33 ± 0.01) and MELN at 200 mg (0.97 ± 0.04 and 2.57 ± 0.13) and 400 mg/kg (1.83 ± 0.04 and 3.98 ± 0.13). The urine volume was significantly (*P* <

Table 1. MELN on urine volume and diuretic activity at 5th h in rats

Groups	Urine volume (ml)	Urinary Excretion (V_0/V_1)×100	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)	pH (1%solution)
Control (25 ml of 0.9% Nacl/kg)	0.66 ± 0.01	18.11	_	_	7.31 ± 0.02
Urea (1 g/kg)	0.83 ± 0.02^{a,c*}	21.56	1.19	_	8.62 ± 0.01^{a*,c*}
Frusemide (5 mg/kg)	2.13 ± 0.01^{a*,b*}	54.34	3.00	2.52	9.25 ± 0.02^{a*,b*}
MELN (200 mg/kg)	0.97 ± 0.04^{a*,b,c*}	25.53	1.41	1.18	8.57 ± 0.03^{a*,c*}
MELN (400 mg/kg)	1.83 ± 0.04^{a*,b*,c*}	48.80	2.69	2.26	9.02 ± 0.06^{a*,b*,c}

Values are expressed as mean ± S.E.M. (Number of animals, n = 6). V_0: Total urinary output; V_1: Total fluid input; UE_t: Urinary excretion in test group; UE_c: Urinary e.xcretion in control group; DA_t: Diuretic action of the test sample; DA_u: Diuretic action of the Urea. a and a* indicates $P < 0.05$ and $P < 0.001$vs. Control, b and b* indicates $P < 0.05$ and $P < 0.001$ vs. Urea, c and c* indicates $P < 0.05$ and $P < 0.001$ vs. Frusemide.

Table 2. MELN on urine volume and diuretic activity at 24th h in rats

Groups	Urine volume (ml)	Urinary Excreti (V_0/V_1)×100	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)	pH (1%solution)
Control (25 ml of 0.9% Nacl/kg)	2.24 ± 0.01	60.27	-	-	7.25 ± 0.01
Urea (1 g/kg)	2.50 ± 0.01^{c*}	64.94	1.08	-	8.54 ± 0.01^{a*,c*}
Frusemide (5 mg/kg)	4.33 ± 0.01^{a*,b*}	110.46	1.83	1.69	8.94 ± 0.02^{a*,b*}
MELN (200 mg/kg)	2.57 ± 0.13^{c*}	67.63	1.12	1.04	8.46 ± 0.02^{a*,c*}
MELN (400 mg/kg)	3.98 ± 0.13^{a*,b*,c}	106.13	1.76	1.63	8.81 ± 0.02^{a*,b*,c}

Values are expressed as mean ± S.E.M. (Number of animals, n = 6). V_0: Total urinary output; V_1: Total fluid input; UE_t: Urinary excretion in test group; UE_c: Urinary excretion in control group; DA_t: Diuretic action of the test sample; DA_u: Diuretic action of the Urea. a and a* indicates $P < 0.05$ and $P < 0.001$vs. Control, b and b* indicates $P < 0.05$ and $P < 0.001$ vs. Urea, c and c* indicates $P < 0.05$ and $P < 0.001$ vs. Frusemide.

0.001) increased in MELN as well as frusemide treated animals comparision with saline treated rats. The MELN at both doses showed dose dependent increase in urine volume in rats. On the basis of urine volume in rats, diuretic activity (Lipschitz value) of standard drug frusemide and MELN at 200 mg and 400 mg/kg calculated were 2.52, 1.18 and 2.26 at 5 h and 1.69, 1.04 and 1.63 at 24 h, which indicates the extracts acts in dose dependent manner.

Effects on electrolytes excretion and pH

The diuretic responses with its electrolyte excretion potency of the extract (MELN) are highly significant in comparison with control animals. MELN increased significantly ($P < 0.001$) the level of Na^+, Ca^{2+} and Cl^- excretion accompanied by the excretion of K^+ at the dose of 200 mg and 400 mg/kg from that of control in dose dependent manner (Table 3 and 4). The urine pH (1% solution) of control animals, 7.31 ± 0.02 and 7.25 ± 0.01 at 5 h and 24 h. Urea was 8.62 ± 0.01and 8.54 ± 0.01 after 5 h and 24 h. Frusemide increased the urine pH 9.25 ± 0.02 and 8.94 ± 0.02 at 5 h and 24 h. MELN at the dose level of 200 and 400 mg/kg, b.w showed 8.57 ± 0.03, 9.02 ± 0.008 and 8.34 ± 001, 8.63 ± 0.01 after 5 h and 24 h, thus making the urine more alkaline.

Effects on natriuretic, saliuretic and carbonic anhydrase inhibition

From the electrolyte excretion of Na^+, K^+ and Cl^- of MELN at both dose levels (200 mg and 400 mg/kg), the natriuretic (Na^+/K^+), saliuretic (Na^+ and Cl^-) activity and carbonic anhydrase inhibition (Cl^-/Na^+ +

Table 3. MELN on electrolytes excretion, saliuretic and natriuretic activity at 5th h in rat

Groups	Electrolytes excretion in mMol/l				$Na^+ + Cl^-$	Na^+ / K^+	$Cl^- / Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25 ml of 0.9% Nacl/kg)	133.43 ± 0.04	55.51 ± 1.15	59.66 ± 0.34	105.33 ± 0.66	238.76 ± 0.70	2.40 ± 0.05	0.5575 ± 0.001
Urea (1 g/kg)	150.26 ± 3.08^{a,c*}	67.50 ± 2.82^{a*,c*}	68.79 ± 0.54^{a,c}	146.66 ± 1.76^{a*,c*}	296.93 ± 4.84^{a*,c*}	2.23 ± 0.05^{a,c}	0.6740 ± 0.010^{a*,b*,c*}
Frusemide (5 mg/kg)	216.76 ± 3.78^{a*,b*}	89.98 ± 0.93^{a*,b*}	78.12 ± 3.24^{a*,b}	176.33 ± 3.17^{a*,b*}	393.09 ± 6.95^{a*,b*}	2.40 ± 0.01^{b}	0.5748 ± 0.001^{b*}
MELN (200 mg/kg)	175.79 ± 1.90^{a*,b*,c*}	72.90 ± 0.84^{a*,c*}	70.50 ± 0.38^{a,c}	151.33 ± 2.40^{a*,c*}	327.13 ± 4.30^{a*,b,c*}	2.41 ± 0.01^{b}	0.6084 ± 0.003^{a*,b*,c}
MELN (400mg/kg)	211.30 ± 2.65^{a*,b*}	87.18 ± 0.59^{a*,b*}	75.83 ± 0.72^{a*,b}	160.66 ± 1.76^{a*,b,c}	371.97 ± 4.27^{a*,b*,c}	2.42 ± 0.01^{b}	0.5383 ± 0.003^{b*,c}

Values are expressed as mean ± S.E.M. (Number of animals, n = 6). a and a* indicates $P < 0.05$ and $P < 0.001$ vs. Control, b and b* indicates $P < 0.05$ and $P < 0.001$ vs. Urea, c and c* indicates $P < 0.05$ and $P < 0.001$ vs. Frusemide

Table 4. MELN on electrolytes excretion, saliuretic and natriuretic activity at 24th h in rats

Groups	Electrolytes excretion in mMol/l				$Na^+ + Cl^-$	Na^+ / K^+	$Cl^- / Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25 ml of 0.9% Nacl/kg)	146.57 ± 0.05	88.58 ± 1.20	96.71 ± 1.02	188.00 ± 1.15	334.57 ± 1.65	1.65 ± 0.01	0.7995 ± 0.001
Urea (1 g/kg)	168.22 ± 1.31^{a*}	101.79 ± 1.64^{a*,c*}	123.93 ± 0.83^{a*,c*}	218.00 ± 1.73^{a*,c*}	386.22 ± 3.04^{a*,c*}	1.65 ± 0.03^{c}	0.8074 ± 0.007^{c*}
Frusemide (5 mg/kg)	170.44 ± 1.21^{a*}	110.51 ± 1.28^{a*,b*}	140.47 ± 1.02^{a*,b*}	242.00 ± 1.15^{a*,b*}	412.44 ± 2.37^{a*,b*}	1.54 ± 0.007^{a*,b*}	0.8614 ± 0.003^{a*,b*}
MELN (200 mg/kg)	163.19 ± 0.76^{a*,b,c}	103.76 ± 0.36^{a*,c}	121.50 ± 0.66^{a*,c*}	221.33 ± 1.76^{a*,c*}	384..52 ± 2.41^{a*,c*}	1.57 ± 0.01^{a*}	0.8291 ± 0.004^{a*,c*}
MELN (400mg/kg)	166.23 ± 0.80^{a*,c*}	108.97 ± 0.39^{a*,b}	136.91 ± 0.50^{a*,b*,c}	236.66 ± 2.90^{a*,b*}	402.90 ± 3.61^{a*,b}	1.52 ± 0.002^{a*,b*}	0.8599 ± 0.007^{a*,b*}

Values are expressed as mean ± S.E.M. (Number of animals, n = 6). a and a* indicates $P < 0.05$ and $P < 0.001$ vs. Control, b and b* indicates $P < 0.05$ and $P < 0.001$ vs. Urea, c and c* indicates $P < 0.05$ and $P < 0.001$ vs. Frusemide.

K^+) were estimated and compared with standard diuretics, urea and frusemide. No carbonic anhydrase inhibition was detected (Table 3 and 4) (Somova *et al.*, 2003). The natriuretic ratio was 2.41, 2.42 and 1.57, 1.52 at the doses of 200 and 400 mg/kg of MELN compared with 2.40 and 1.54 of frusemide at 5 h and 24 h respectively. The saliuretic effect was 327.13, 371.97 and 384.52, 402.90 of 200 and 400 mg/kg comparable with 393.09 and 412.44 of frusemide at 5 h and 24 h respectively.

DISCUSSION

Diuretics are drugs that increase the rate of urine flow and sodium excretion and are used to adjust the volume and/or composition of body fluids in a variety of clinical situations, including hypertension, heart failure, nephritic syndrome and cirrhosis. Loop diuretics such as frusemide can increase the urinary flow rate; also they are strongly saliuretic in as much as they increase urinary sodium and chloride excretion (Jackson, 2001) that is why in this study frusemide was used as standard drug. Frusemide used in this experiment belongs to the loop or high ceiling diuretics, which act by inhibiting $Na^+ / K^+ / 2Cl^-$ cotransport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing

Na^+ and Cl^- from the body (Das *et al*., 2005). On the other side, the herbal diuretics produce very little acute toxicity and in general they can be considered as mild and good drugs, in comparison to other diuretics used now a day in therapeutic.

The most important thing is that a large number of these plants are rich in potassium, which would not lead to potassium depletion (Horisberger and Giebisch, 1987). Thirty to seventy percent of the K^+ filtered by the glomerules is known to be reabsorbed by the proximal convoluted tubule (Stanton and Giebisch, 1992) by a combination of three processes: active transport, paracellular diffusion and solvent drag (Wilson *et al*., 1997). It was reported that an increment of the urine output in rats might result from high potassium content in the plant extract (Nilveses *et al*., 1989). The increased Na^+ concentration that reaches the distal tubule results increased loss of K^+. There is an increase in the excretion of Ca^{2+} (Rang *et al*., 2003).

Diuretic has two separate connotations, increase in urine volume and net loss of solute (i.e. electrolytes) and water (i.e. saliuretic effect) (De Stevens, 1963; Jackson, 1996). These two processes are involve in the suppression of renal tubular absorption of electrolytes, water and low molecular weight organic compounds into the blood stream, and as a consequence, promoting the formation of urine (De Stevens, 1963; Milton *et al*., 1970).

The research work was performed on the basis of its folkloric use as a diuretic. From this work, *Lippia nodiflora* induced an increment in the urine output and in the electrolyte excretion at both doses of the extract at 5^{th} h and 24^{th} h in normal rats. In the 5^{th} h and 24^{th} h, the MELN extracts showed change in urine output at both dose levels tested (200 and 400 mg/kg), the diuretic effect of the methanol extracts was significant at 5 h and 24 h. However, there was moderately delayed effect at 24^{th} h, even though, the diuretic activity at 24^{th} h at both doses was significant. It showed the extracts act in dose dependent manner. These results reveal that the methanol extract of *Lippia nodiflora* is more potent diuretic; the water excretion is higher in the presence of this extract.

It was noted that MELN treatment caused increase in both water and electrolytes excretion qualitatively similar to frusemide, which is known by its potential saliuretic and diuretic effects (Leuschner, 1995). The extract significantly increased the volume of urine with considerable Na^+ and Cl^- load, which was comparable to that of urea and frusemide.

MELN increased significantly urine flow rate and maintain the alkaline pH as compare with control and more/less similar to frusemide. It is possible that MELN extract exerted its diuretic activity by inhibiting tubular reabsorbtion of water and accompanying anions, as such action has been hypothesized for some plants (Pantoja *et al*., 1991; Bevevino *et al*., 1994). As it was emphasizied, diuretic effect of MELN could be due to active phytoconstituents such as flavonoids, saponins and terpenoids (Rizvi *et al*., 1980; Sood *et al*., 1985; Chodera *et al*., 1991). The preliminary phytochemical analysis revealed that these compounds mainly present in the MELN.

In the toxicological evaluation, this plant did not exhibit any toxic effects up to 2,000 mg/kg, b.w. From the experiment, *Lippia nodiflora* can be considered as nontoxic, because there is no mortality up to 2,000 mg/kg.

In conclusion, the present results demonstrate that *Lippia nodiflora* (Verbenaceae) induces significant effects on urinary excretion of water and electrolytes. It shows that the active phytoconstituents in MELN had similar diuretic spectrum to that of frusemide. The further work is in progress to find out the active principles responsible for diuretic activity.

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