

EVALUATION OF DIURETIC ACTIVITY OF MILK EXTRACT OF *SEMECARPUS ANACARDIUM* NUT

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ABSTRACT

Semecarpus anacardium 'Marketing Nut' is a tropical flowering plant widely distributed in India. *Semecarpus anacardium* are widely used for its therapeutic potential as immunomodulatory and in folklore the plant material is reported in the treatment of diuretic. Since, the diuretic activity of these plant materials has not been investigated in scientifically controlled studies; the aim of the present study was to evaluate the diuretic potential of milk extracts of *Semecarpus anacardium* seed in normal rats after acute and sub-chronic oral administration. Milk extracts of *Semecarpus anacardium* seed (150 and 300 mg/kg) along with the reference drug, furosemide (10 mg/kg) were administered orally to male Wistar rats for 8 subsequent days and their urine output was quantitated at several intervals of time after the dose. Several other parameters such as urinary electrolyte concentration, plasma electrolyte concentration and creatinine clearance were also measured by flame spectrophotometry and Jaffe alkaline picrate method. After administration of single dose of the milk extracts of *Semecarpus anacardium* seed, urine output significantly showed equivalent increment at all time points, and at 24 h after the dose, the total volume of urine excreted was highest in furosemide followed by plant extracts and control group. Both extracts increased the urinary levels of Na⁺ and K⁺, to about the same extent, while furosemide increased urinary levels of only Na⁺ and decreased urinary K⁺. Despite changes in urinary excretion of the electrolytes, plasma Na⁺ and K⁺ levels were not affected by any of the substances. The present study supports the ethnomedical use of *Semecarpus anacardium* seed for their diuretic effect

Keywords: *Semecarpus anacardium* seed, Milk extracts, Furosemide, Diuretic activity, Urine output, Plasma Na⁺ and K⁺ levels.

INTRODUCTION

Diuretics are the agents which causes increase in excretion of urine. These drugs generally used in the treatment of oedema, hypertension, and congestive heart failure (CHF), Nephritis, toxemia and other UTI disorders. Diuretics are also used in the treatment of pulmonary congestion and play vital role in pregnancy and premenstrual tension.¹ Presently in market synthetic diuretics are available which are having significant side effects. These synthetic diuretics significantly inhibit K⁺ secretion and leads to K⁺ retention². Plant medicine is commonly used in the traditional treatment of some renal diseases, and many plants are reported to possess significant diuretic activity. The diuretic activity of a number of plants used in ethnomedicine as diuretic agents has been confirmed in experimental animals.³

In the present study we selected a plant namely *Semecarpus anacardium* (Linn.) belonging to the family of Anacardiaceae. It is distributed in the sub-Himalayan tract from the Bias eastwards, ascending in the outer hills up to 3,500 ft., Assam, Khasia hills, Chittagong, Central India and the Western Peninsula. The fruit and seed is acrid in taste, hot, sweetish. In traditional system of medicine it is used as a digestible, aphrodisiac, anthelmintic laxative. It also used treat skin diseases, piles, dysentery, tumors, fevers, loss of appetite, urinary discharges, heals ulcers, and strengthens the teeth, useful in insanity, asthma. The oil is tonic, makes hair black, good for leucoderma, coryza, epilepsy and other nervous diseases. It lessens inflammation, useful in paralysis and superficial pain⁴

Earlier the plant has been studied for its analgesic and anti-inflammatory⁵, antiarthritic⁶, antimicrobial⁷, antibacterial⁸, anthelmintic⁹, antimutagenic¹⁰, antidiabetic¹¹, antitumour¹², antioxidant¹³, fungistatic¹⁴, hepatocellular carcinoma¹⁵⁻¹⁷, hypocholesterolemic¹⁸, hypolipidemic¹⁹, immunomodulatory²⁰ and mammary carcinoma²¹ activities.

MATERIAL AND METHODS

Plant Material Collection

Seeds of plant were collected from local regions of Uttar Pradesh, and the plant was authenticated as *Semecarpus anacardium* by the Dr. A.K.S. Rawat, National Botanical Research institute(NBRI), Lucknow Campus. A voucher specimen (Specimen No: NBRI/CIF/328/2012) is preserved in NBRI, LUCKNOW.

Preparation of milk extract²²

S. anacardium Linn. nut extract contains purified nuts of *S. anacardium* and cow's milk in the ratio as indicated in the Formulary of Siddha Medicine. 200 g of the nut was boiled with 500 mL of milk, which was repeated thrice. The decoction was stored at room temperature and this was used for the study.

Experimental Animals

Male Wistar rats about 150 - 200 g were used for study. All animals were housed in a group of 6 in polyethylene cages under standard housing conditions (12:12h light and dark cycle, temperature 22±2°C and humidity 50±10%) with standard feed pellet and free access to water *ad libitum*. Standard hygiene conditions were maintained. The animal experiments were performed in accordance with our Institutional Animal Ethics Committee (IAEC/APPC/02/12) and by the animal regulatory body of the government. After two weeks of acclimatization, animals were used for the following studies.

Standard drug

Furosemide (Lasix, Aventis Pharma Limited, India), a high-ceiling loop diuretic, was used as the reference drug (positive control). It was dissolved in water for injection prior to administration.

Biochemical methods

Blood was collected by retro-orbital puncture under light diethyl ether anesthesia. Plasma was obtained by centrifugation (6000 rpm at 7-10 min) and for urine (3000 rpm for 10 min) were analyzed. Plasma and urinary levels of sodium and potassium were quantified by flame spectrophotometry.

Experimental Design

Evaluation of diuretic activity

The assessment of diuretic activity was evaluated as follows: 24 animals were placed in separate metabolic cage 1 day prior to actual experiment for acclimatization. These animals were divided into four groups of six rats each for the study. Rats were subjected under fasting condition overnight with free access to water and treatment was followed in the manner described below.

Diuretic activity

Prior to initiation of the experiment, each animal received physiological saline (0.9% NaCl) at an oral dose of 5 mL/100 g body weight, to impose a homogenous water and salt load²³. The **first group** (Vehicle control) received orally distilled water 10 mL/kg body weight. The **second group** (Standard) was treated with intraperitoneal administration of furosemide with a dose of 10 mg/kg body weight. **Third and fourth group** were administered orally 150 and 300 mg/kg body weight of *Semecarpus anacardium* seeds extract respectively. Urine was collected and measured at 1, 2, 4, 6, and 24 h after the dose. Sodium and potassium concentrations were estimated in the 24 h urine samples as well as in the plasma of rats.^{24, 25, 26}

Table 1: Effect of single dose of the root extract (150 mg and 300 mg) of *Semecarpus anacardium* and furosemide on 24 h urinary excretion

Groups	Urine volume (ml), After 1hr.	Urine volume (ml), After 2hr.	Urine volume (ml), After 4hr.	Urine volume (ml), After 6hr.	Urine volume (ml), After 24hr.
Group I	1.82±0.029	2.82±0.068	3.17±0.075	4.89±0.082	7.71±0.096
Group II	4.35±0.136***	7.44±0.131***	9.28±0.170***	12.67±0.25***	16.96±0.317***
Group III	2.18±0.07 ^{bs}	3.33±0.069**	5.37±0.183***	6.767±0.29***	8.70±0.137***
Group IV	2.30±0.131*	5.89±0.098***	7.09±0.151***	9.8±0.21***	12.92±0.223***

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's "t" test. Number of animals in each group n = 6. a Comparison made with vehicle control group; b Comparison made with CRS group. ***P<0.001; **P<0.01

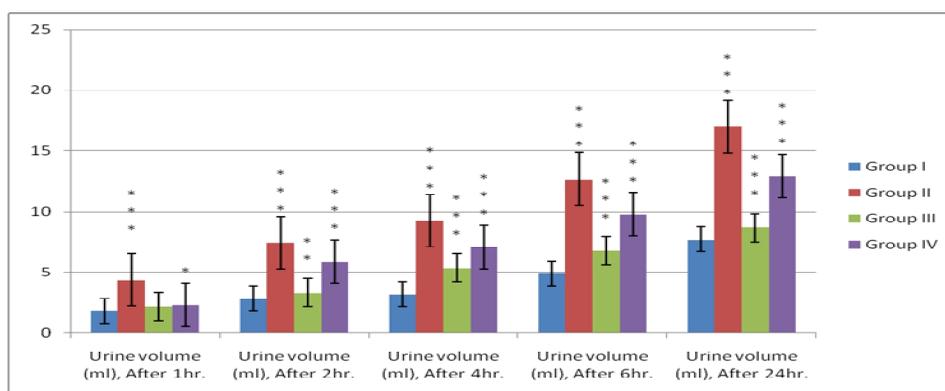


Figure 1: Effect of single dose of the root extract (150 mg and 300 mg) of *Semecarpus anacardium* and furosemide on 24 h urinary excretion

Table 2: Effect of the milk extract of plant, and furosemide on 24 h urinary electrolyte excretion and plasma Na⁺ and K⁺ levels in normal rats

Treatment	Dose(mg/kg BW)	Urinary Electrolyte Conc.		Plasma Electrolyte Conc.	
		Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)
Water	10	73.6±1.5	60±103	143.56±2.8	4.9±0.3
Furosemide	10	103.4±2.8***	52.1±1.3**	137±1.6	5±0.4
Extract 1	150	117.4±2.3*	63.1±2.4**	135.3±1.0	4.8±0.3
Extract 2	300	140±5.3***	73.2±0.84***	133.9±0.5	5.2±0.2

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's "t" test. Number of animals in each group n = 6. a Comparison made with vehicle control group; b Comparison made with CRS group. ***P<0.001; **P<0.01

RESULTS

Effect on urine volume

At first, the study with single dose of milk extract (150 and 300 mg) showed increased diuresis (Figure 1), which subsequently showed rise in urine output on comparison with control group rats at an interval of 4h after dose (Milk extract 150 mg (5.37±0.18 mL) 300 mg (7.09±0.15 mL) v/s control group 3.17±0.07 mL). Urine output showed simultaneous increase over the period of experimental study, such that the cumulative urinary excretion was significantly higher at 6 h (Milk extract 150 mg (6.76±0.2 mL) 300 mg (9.8±0.2 mL) v/s control group 4.89±0.8 mL) and 24 h (Milk extract 150 mg (8.7±0.13 mL) 300 mg (12.9±0.2 mL)

v/s control group 7.7±0.09 mL) (Figure 1). The effect of a single dose of the reference diuretic, furosemide, was also rapid and higher than that of the plant extracts (Figure 1). Reference drug showed the highest value in terms of urine output measurement (furosemide 16.9±0.3 mL).

Effect on urinary electrolyte excretion

The effect of furosemide and the milk extracts of *Semecarpus anacardium* on electrolyte (Na⁺ and K⁺) excretion in the 24 h urine is presented in Table 1. The milk extract of plant potentiated the electrolyte excretion [Na⁺ (P < 0.001) and K⁺ (P < 0.05)], which accounted for greater results than that produced by furosemide, especially that of K⁺. Furosemide

actually decreased K^+ excretion as compared to the controls, such that the Na^+/K^+ excretion ratio (1.98) was higher than for the aqueous extracts (150 mg 1.86, 300 mg 1.91) (Table 2).

Effect on plasma electrolyte levels

On contrary to urinary electrolyte levels, plasma electrolyte levels showed minimal effect on the furosemide and aqueous extract levels of Na^+ and K^+ (Table 2).

DISCUSSIONS

The present study is emphasised on the elucidation of *Semecarpus anacardium* as potent diuretic, as reported in various traditional folk use. Oral administration of the milk extract was used. The pharmacological responses produced were compared to furosemide, a famous diuretic used in clinical therapeutics. The studied accompanied useful evaluation of urine output, urinary electrolyte excretion, plasma electrolyte excretion.

Diuresis is an increase in the production of urine by the kidneys, which typically results in a corresponding increase in urine expelled by the body; diuresis without an accompanying increase of urination can cause severe medical problems. Furosemide acts by inhibiting NKCC2, the luminal Na-K-2Cl symporter in the thick ascending limb of the loop of Henle. The action on the distal tubules is independent of any inhibitory effect on carbonic anhydrase or aldosterone; it also abolishes the corticomedullary osmotic gradient and blocks negative as well as positive free water clearance. By inhibiting the transporter, the loop diuretics reduce the reabsorption of NaCl and also diminish the lumen-positive potential that derives from K^+ recycling. This electrical potential normally drives divalent cation reabsorption in the loop, and by reducing this potential loop, diuretics cause an increase in Mg^{2+} and Ca^{2+} excretion. Prolonged use can cause significant hypomagnesaemia in some patients. Since Ca^{2+} is actively reabsorbed in the distal convoluted tubule, loop diuretics do not generally cause hypocalcaemia.²⁷

The above study of the milk extract of *Semecarpus anacardium* seeds (150 and 300 mg/kg B.W) increased diuresis when compared with control. At an interval of 4h, 6h and 24h urine output of control gp was 3.17, 4.89 and 7.71 mL that differed markedly from extracts 150 mg (5.37, 6.76 and 7.7 mL) and 300 mg (7.09, 9.8 and 12.9) respectively, though furosemide resulted in quantitatively highest collection of urine i.e. 9.28, 12.67 and 16.9 mL at 4h, 6h and 24h on day 1. Quantification of urinary and plasma electrolyte was measured using flame spectrometry and units were expressed in mmol/L. As per analysis values of Na^+ in plasma (Control 143.5 mmol/L, Furosemide 137 mmol/L, milk extract 150 mg 135.3 mmol/L and milk extract 300 mg 133.9 mmol/L) were higher on comparison with urine (Control 73.6 mmol/L, Furosemide 103.4 mmol/L, milk extract 150 mg 117.4 mmol/L and milk extract 300 mg 140 mmol/L) and subsequently values of K^+ in plasma (Control 4.9 mmol/L, Furosemide 5 mmol/L, milk extract 150mg 4.8 mmol/L and milk extract 300 mg 5.2 mmol/L) showed steep downfall on comparison with urine (Control 60 mmol/L, Furosemide 52.1 mmol/L, milk extract 150 mg 63.1 mmol/L and milk extract 300 mg 73.2 mmol/L).

CONCLUSIONS

In conclusion, the present studies support the ethnomedical use of *Semecarpus anacardium* for their diuretic effect. The diuretic activity of the plant appeared to be mainly related

with the salt concentrations as a metabolite in the plant process. This generally rules out the osmotic phenomenon as there was an increase in sodium levels and there by decrease in the other salt. Plant extracts does not seem to have any renal toxicity subjected to the experimental condition. Based on the pattern of excretion of water, sodium and potassium, it appears that there is at least a single active principals present in these extracts, which is having a furosemide-like activity. These findings suggest, for the first time, mechanism of diuretic action of *Semecarpus anacardium* used in traditional medicine.

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