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## **Evaluation of diuretic activity of root extract of *Mangifera indica* in albino rats: An original research study**

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**Abstract**---Background: Diuretics increase urine flow and sodium excretion are used to maintain the volume and composition of body fluids in a variety of clinical situations Objectives: To evaluate the diuretic activity of alcoholic (AERMI) extract of roots of *Mangifera indica* in albino rats. Methods: 5 groups of Albino rats were used to evaluate the diuretic activity by using metabolic cages. The group I serves as normal control received vehicle, the group II received Furosemide (10 mg/Kg, p.o) ; other groups III, IV, V were treated with low, medium, and high doses of AERMI and immediately after extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in metabolic cages. Total volume of urine collected was measured at the end of 5 h. Concentration of Sodium, Potassium, Chloride in the urine were estimated. Results: Compared to control group the AERMI at different dose levels (100,200 and 400 mg/kg) has significantly

increased the urine volume and also enhanced the elimination of Sodium, Potassium and Chloride. Conclusion: Single dose of AERMI as 100, 200 and 400 mg/Kg and Frusemide have increased the urine output along with an increase in concentration of Sodium, Potassium, and Chloride.

**Keywords**--M. indica, roots, alcoholic extract, hydrated rats, diuretic.

## Introduction

Diuretic compounds that stimulate the excretion of water are potentially useful in most of disorders including those exhibiting edema Such as congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension<sup>1</sup>,hypertension.The presently available diuretics such as thiazides and loop diuretics exhibit various adverse effects such as electrolyte imbalance,metabolic alterations<sup>2,3</sup>.vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as *Abelmoschus esculentus*, *Bacopa monnieri*, *Barbara vulgaris*<sup>2</sup> and *Cissampelos pareira*, *Mangifera indica*<sup>4,5</sup>.

## Plant description<sup>4,5</sup>

*Mangifera Indica*, also called as mango, aam, it is a significant plant which was using since from 4000 years in traditional and indigenous medical system. *Mangifera* plant belongs to the family of Anacardiaceae. According to traditional medicinal system, mangifera contains different medicinal properties. Various parts of mango palnt are used therapeutically as a antioxidant<sup>7</sup>, antidiabetic<sup>6</sup>, antiseptic, astringent, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles. All parts are used to treat tumour, snakebite, stings, datura poisoning, heat stroke, miscarriage, anthrax, blisters, wounds in the mouth, tympanitis, colic, diarrhea, glossitis, indigestion, bloody dysentery, liver disorders, excessive urination, tetanus and asthma.

Ripe mangifera fruit is considered to be revitalizing and refreshing. The juice is used as restorative tonic in heat stroke. The mango seeds are used in asthma and as an astringent. Vapours of the burning roots are inhaled to relieve hiccups and different afflictions of the throat. The stem bark of mangifera possess strong astringent action, it is also useful in conditions of diphtheria, rheumatoid arthritis and syphilis. The gum like substance obtained from this plant is used in dressings for fractured feet and for scabies. The main aim of the present study was to evaluate the diuretic activity of alcoholic extract of roots of *Mangifera indica* in hydrated rat model (Modified Lipschitz test ) in albino rats.

## Collection of plant

The roots of *M.indica* were obtained from mango farm in Vizianagaram,Andhra Pradesh and were identified and authenticated by Dr. Satish Kumar, Botanist Government degree college ,Vizianagaram ,Andhra Pradesh,India.

### **Preparation of extract**

Roots were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation of alcoholic extract of roots of *M.indica* was done by using Soxhlet apparatus<sup>8</sup>. About 200 g of roots powder was taken into the Soxhlet apparatus and extracted using (95%) ethanol. The extraction process was carried out for 18 - 20 h till the appearance of colourless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at <50° C and percentage yield of ethanolic extract of roots of *M.indica* was recorded with respect to the total quantity of powder used for the extraction. Then the extract was evaluated for its phytochemicals by following standard procedures<sup>14</sup>.

### **Experimental Animals**

Male Albino rats (36) weighing between 140-200 g used in the study (5 groups; n = 6) were obtained from the Central Animal House, Maharajah's Institute Of Medical Sciences, Vizianagaram, Andhra Pradesh, India. The experimental protocol was approved by the Institutional Animal Ethical Committee. The animals were maintained under standard husbandry conditions temperature 22±2°C, humidity 45-55%, light: dark cycle (12:12h) for an acclimatization period of 15 days before performing the experiments. All rats were placed in metallic cages 3 in each.

Drugs used: Furosemide 20 mg/ml (Sanofi Aventis, Andheri East, Mumbai.)

### **Acute toxicity study<sup>9,10</sup>**

Determination of LD<sub>50</sub> : The acute toxicity of AERMI was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 h study period (short term toxicity). Based on the short term toxicity profile, the next dose of the extract was determined as per OECD guidelines No.420. The maximum dose tested (2000 mg/kg) for LD<sub>50</sub>. From the LD<sub>50</sub>, doses like 1/20<sup>th</sup>, 1/10<sup>th</sup> and 1/5<sup>th</sup> were selected and considered as low, medium and high dose i.e: 100 mg/kg, 200 mg/kg, 400 mg/kg respectively to carry out this study.

### **Experimental Model**

#### **Lipschitz Test<sup>11,12</sup>**

Male Albino rats were divided into 5 groups of 6 rats in each. The I group serves as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt), the II group received Furosemide (10 mg/Kg, p.o) in vehicle; other groups III, IV, V were treated with low, medium, and high doses of AERMI in vehicle and immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5h was measured at the end. During this period no food and water was made available to

animals. Various parameters like total urine volume and concentration of Sodium, Potassium and Chloride in the urine were measured and estimated respectively.

### **Estimation of urinary electrolytes**

Urine electrolytes (sodium, potassium and chloride) were determined by Ion Selective Electrode method as described by the user instruction manual of the biochemical kits (Roche, Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana.)

### **Statistical Analysis**

Experimental results were expressed as mean + SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnetts 't' test.

### **Results**

The AERMI was subjected to qualitative phytochemical tests to identify the phytochemical constituents and it confirmed the presence of alkaloids, sterols, phenolic compounds, tannins, flavonoids and resins. In acute toxicity tests all the rats were survived even after 14 days. This denotes that the AERMI was found to be safe up to the maximum dose level tested (2000 mg/kg). Major behavioural changes were not observed during this study period. The results obtained with assessment of diuretic activity of AERMI was shown in Table No.1. From the result it can be observed that AERMI has shown a potential diuretic activity by increasing urinary out put and increased excretion of sodium ,potassium, chloride levels when compared to control. The effect of AERMI was found to be dose dependent, i.e, among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide, the diuretic effect observed after treatment with AERMI was found to be significant in terms of urinary output, sodium, potassium, chloride concentrations. Determination of urinary electrolyte concentration revealed that AERMI was effective in increasing urinary electrolyte concentrations for all the three ions tested ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ).

### **Discussion**

Medicinal plants offer a natural protection against diseases and are a substantial treatment for certain disorders. Diuretics have proved to be extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral oedema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnoea<sup>13</sup> in CCF and acute left ventricular failure. They decrease plasma volume and subsequently venous return to the heart. This decreases the cardiac work load, oxygen demand and plasma volume and also decreases blood pressure. Thus diuretics play an important role in hypertensive patients<sup>14</sup>. They are used to induce forced diuresis (forced alkaline diuresis and forced acidic diuresis) in cases of aspirin and morphine poisoning. Diuretics are also useful in prevention of recurrent calculi. The present study revealed that AERMI significantly increased the urinary out, as well as the

elimination of urinary electrolytes in a dose dependant manner. Earlier Hullatti et al, 2011 and Suresh Babu Sayana et al,2014 reported diuretic activity with methonolic and alcoholic extracts of roots of *C.pareira*<sup>3,17</sup>. In the present study alcoholic extract of roots of *M.indica* was studied for its diuretic activity. The phytochemical<sup>15</sup> studies reveals that the roots of *M.indica* contains flavanoids, alkaloids, carbohydrates, sterols, phenolic compounds, tannins, resins. Earlier studies reported phytochemical substances like flavonoids, saponins, organic acids<sup>17,2</sup>, steroids, carbohydrates, tannins, phenolic compounds, terpenoids, alkaloids<sup>17</sup>, glycosides<sup>18</sup>, sterols, sesquiterpenes & aminoacids, carotinoids<sup>19</sup> in different plant extracts. AERMI was identified with most of these plant phytochemical substances mentioned above. Hence it can be reported that the observed diuretic activity is due to these above phytoconstituents.

### Conclusion

Results showed that a single dose administration of AERMI as 100,200 and 400 mg/Kg and standard Frusemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium, Potassium, and Chloride in urine. AERMI 400 mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide(10 mg/kg).The present study supports and justify the rationale behind the folklore use of root of *Mangifera indica* for diuretic activity.

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Table No: 1 Effect of AERMI on urine volume and electrolyte concentration in hydrated rat model (mean  $\pm$  SEM)

S.No	Treatment	Total Urine Vol (ml/kg/5 h)	Conc of Na <sup>+</sup> (mmol/L)	Conc of K <sup>+</sup> (mmol/L)	Conc of Cl <sup>-</sup> (mmol/L)
1.	Control (10ml/Kg )	3.52 + 0.08	111.02 + 2.14	50.08 + 1.41	81.85 + 1.38
2.	Standard(Frusamide 10mg/kg)	10.12+ 0.10***	182.51+2.22** *	86.82+ 1.50***	128.05+ 1.57***
3.	AERMI Low (100mg/kg)	4.10 + 0.06***	126.30+2.70** *	63.13+ 1.72***	93.32 + 1.72***
4.	AERMI Medium(200mg/kg )	5.86 + 0.05***	162.99+2.00** *	76.93+ 2.57***	108.44+ 1.19***
5.	AERMI High(400mg/kg )	8.12 + 0.08***	190.05+2.08** *	84.11+ 1.69***	120.38+ 2.00***

n=6, Significance at \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, Compared with control group (One Way ANOVA followed by Dunnetts t test)