STUDY DIURETIC ACTION OF LEAVES OF MANGIFERA INDICA

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ABSTRACT
A dried powder of Mangifera Indica is substituted for extraction using ethyl acetate, 60% ethanol, and water in upper order to polarity prepared. Mangifera Indica L. indicates the presence of alkaloids, tannins, saponins, steroids, flavonoids, terpenes by phytochemical analysis of ethanolic extract. Then diuretic activity tested by Aqueous and 60% ethanolic extract of Mangifera Indica. Diuretic activity tested by Lipchitz method. In toxicity studies 200mg/kg & 400 mg/kg dose was selected for given activity. After 24 hours urine output was calculated. In the given study Furosemide (20mg/kg) served as a positive control by giving IP route and by giving IV route and by oral route extracts were given. Na⁺, K⁺, Cl concentration in urine was determined by flame photometer instrument. 60% ethanoic extract and aqueous extract of Mangifera Indica L. leaves at 400 mg/kg have increased the urine output significantly. These extracts of 60% EEMI & AEMI at 400 mg/kg b.w. has shown better diuretic activity, whereas moderate diuretic activity was shown at 200 mg/kg dose. From current study, it is concluded that aqueous extract and 60% ethanolic extract of Mangifera Indica L. has diuretic and natriuretic activity.

KEYWORDS: Mangifera Indica L., Na⁺/ K⁺ ratio, Diuretic, Photochemical.

INTRODUCTION
Mangifera Indica L, commonly known as mango, from family Anacardiaceae, formed all over the world and it consists of tropical fruit-bearing trees. Mangifera Indica L. is rich in secondary metabolites. Mango is the National fruit of India. Also found in Pakistan and Bangladesh. An unripe fruit of Mangifera Indica L. is Antiascorbic and ripe fruit have apoplexy use. Leaves are useful in Antibacterial, diuretic and also have used in Diabetes.
Mellitus. Stem bark is used as Astringent and used for diarrhea. The kernel is used as astringent, anti-inflammatory, antifungal, antibacterial, anthelmintic, the antispasmodic activity also gives in the treatment of diarrhea, diabetes, and menstrual disorders. Leaves Of given Mangifera Indica L. are useful in a cough, burning sensation, wounds, ulcers, diarrhea, dysentery. Leaves of Mangifera Indica L. contains, saponins, glycosides, unsaturated sterols, polyphenols, Mangiferin and tannins, lupeol, and beta-sitosterol. Mangiferin is the main constituent present in leaves and twigs.[1] On literature review, it is found that traditional use of Mangifera Indica is for diuretic activity. The Plant contains Alkaloids, saponins, tannins, steroids, flavonoids, polysaccharides, proteins and amino acids.

There are different pharmaceutical activities which are Antibacterial and antioxidant activity[2] Antimycobacterial and immunomodulatory activity.[3] antibacterial activity.[2] Hemolytic activity.[5] Etc. However, the current study of Mangifera Indica L. indicates Diuretic Activity at different concentration of ethanolic extracts.

**SCIENTIFIC CLASSIFICATION**

1. Kingdom: Plantae
2. Clade: Angiosperms
3. Family: Anacardiaceseous
4. Genus: Mangifera
5. Species: M. indica
6. Binomial name: Mangifera indica L.

“Fig.1 Mangifera Indica L. Tree”.

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**Fig.1 Mangifera Indica L. Tree**.
MATERIALS AND METHODS
Leaves of Mangifera Indica L. were collected from Dr. Vikhe Patil College of pharmacy, Ahmednagar. Ethanol and Methanol were obtained from Rajas Chemicals, Rahuri, Ahmednagar.

Preparation of Plant Extracts
The dried powder of leaves of Mangifera Indica L. plant extracted with 60% ethanol, ethyl acetate, and water by soxhlet extraction Method. After complete extraction solvent was removed from the extract by Rota- flash evaporator. at room temperature, the crude extracts of Mangifera Indica L. were dried. All the extracts were transferred to a decontaminated container and then kept in a freezer.

Preliminary Phytochemical Screening
Leaf extracts of 10% W/V solution is used to identify the presence of various secondary metabolites in the given extracts were used for detection of the presence of different phytoconstituents.[7] Identification of Secondary metabolites is alkaloids, tannins, sterols, Polysaccharide’s, proteins and amino acids, etc.is given in table no 1.

Experimental animals
From Department of Pharmacology of Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar experimental Albino Mice and rats were taken. At 27 c +- 2%, these entire animals were put in standard polypropylene cages under 12 hour’s dark/light cycle. The animal experiments were approved by the Institutional animal ethics committee (IAEC) of Dr. Vitthalrao Vikhe Patil Foundation’s College of Pharmacy, Ahmednagar.
Toxicity study

Study of toxicity of ethanol extract, ethyl acetate and water extract of leaves of Mangifera Indica L. were determined as per CPCSEA guideline no. 420 (Fixed Dose Method). Mice are divided into 8 groups of 6 animals each. By Oral Route, a normal saline solution will receive as 5 ml/kg body weight. Other groups of 50, 100, 200, 400, 800, 1000, 2000, 4000 mg/kg b.w. of extract will orally give. After 4 hours, animal behavior was observed.[9,10]

Reference and Standard Drug

Furosemide (20mg/kg b.w) was used as Reference standard (Positive Control) IP. (Sanofi India Limited) And control drug used is Normal Saline Solution (50 ml/Kg).

Study of Diuretic Activity[11,12]

For the study of diuretic activity, Lipchitz Method was used. For Diuretic activity, then rats are put on 24 hours fasting. Every 6 Rats are classified into 6 groups.

Group 1 is Control group and normal saline is given orally (25ml/kg), Group 2 is a standard group and the same amount of normal saline is given by intraperitoneal route in which 60%, different concentration of ethanolic extracts of Mangifera Indica L. (60%) and Aqueous extract of Mangifera Indica L. (AEMI) are liquefied.

Animals of Group 3 received 200mg/ kg of 60% ethanolic extract of Mangifera Indica L., Group 4 animals given 400 mg/ kg of 60% EEMI, 200mg/kg of AEMI was given to Group 5 and finally, animals of group 6 were given of 400mg/kg AEMI.

For administration, freshly prepared drugs are used. Each preparation is given in form of fluid in all cases in the same way. at end of 24 hours, the urine was collected and calculated.

Study of Urine Analysis

Flame photometer is used for determination of the concentration of Na⁺, K⁺, Cl⁻ in a urine sample.

Calculation of Diuretic considerations

a) Diuretic Index: \( V_t/V_c \)

b) Lipchitz Value: \( V_t/V_r \)

c) Saluretic Index: \( C_t/C_c \)

d) Na+/K+ ratio: \( C_n/C_k \)
Where,

$V_t$: urine volume of test group.

$V_c$: urine volume of the control group.

$V_r$: urine volume of the reference group.

$C_t$: concentration of electrolyte in the urine of test group.

$C_c$: concentration of electrolyte in the urine of control group.

$C_n$: concentration of sodium ion in the urine of group &

$C_k$: concentration of potassium in the urine of the same group.

**RESULTS**

In leaves of leaves Mangifera Indica L. plant which expressively shows the presence of tannins, flavonoids, phenols, sterols, alkaloids, tannins, polysaccharides and terpenes. In together, 60% aqueous extract also an ethanolic extract of traces of sterols, proteins, and amino acids were present, but not in methyl acetate extract. So, ethanolic extract of Mangifera Indica L. of 60% and Aqueous extract of Mangifera Indica L. were selected for existing study. In toxicity study, It was determined that administration of ethanolic extract of Mangifera Indica L. (60% Concentration) and aqueous extract of Mangifera Indica L. up to 5 gm./ kg by weight does not produce any adverse effect on animals. Hence, for diuretic activity, 1/20th and 1/10th Dose (200 mg/kg and 400 mg/kg) were selected. and All 60% concentration of ethanolic extract of leaves of Mangifera Indica L. shows potential Diuretic activity.

**Table 1: Physicochemical evaluation of Mangifera Indica L. leaf extracts.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Types of Phyto Constituents</th>
<th>Ethyl Acetate Extract</th>
<th>50% Ethanol Extract</th>
<th>70% Ethanol Extract</th>
<th>90% Ethanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Polysaccharides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Proteins &amp; Amino Acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2: Diuretic Activity of Mangifera Indica L. Leaf Extract.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Dose</th>
<th>Urine Volume</th>
<th>Conc. Of electrolyte (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conc. Of Na+</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>Saline 25ml/kg p.o</td>
<td>4.5ml</td>
<td>170 +/- 1.6</td>
</tr>
<tr>
<td>2.</td>
<td>Standard</td>
<td>20mg/kg b.w. i.p</td>
<td>8.0ml</td>
<td>306.2 +/- 2.0***</td>
</tr>
<tr>
<td>3.</td>
<td>60% EEMI</td>
<td>200 mg/kg p.o</td>
<td>4.0ml</td>
<td>185.5 +/- 3.4***</td>
</tr>
<tr>
<td>4.</td>
<td>60% EEMI</td>
<td>400mg/kg p.o</td>
<td>5.0ml</td>
<td>195.5 +/- 1.6***</td>
</tr>
<tr>
<td>5.</td>
<td>AEMI</td>
<td>200 mg/kg p.o</td>
<td>4.3ml</td>
<td>223.7 +/- 1.8***</td>
</tr>
<tr>
<td>6.</td>
<td>AEMI</td>
<td>400mg/kg p.o</td>
<td>6.0ml</td>
<td>248.8 +/- 1.6***</td>
</tr>
</tbody>
</table>

Values of +/- S.E.M. of six rats / Treatment.

***P<0.01, ***P<0.001 vs. Control

Table 3: Evaluation of Diuretic Parameters.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Dose</th>
<th>Na+/K+ Conc.</th>
<th>Lipschitz Value</th>
<th>Diuretic Index</th>
<th>Na+/K+ Saluretic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>Saline 25ml/kg p.o</td>
<td>2.05</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>Standard</td>
<td>20mg/kg b.w. i.p</td>
<td>2.60</td>
<td>1.05</td>
<td>2.60</td>
<td>1.90/1.40</td>
</tr>
<tr>
<td>5.</td>
<td>70%EEMI</td>
<td>200 mg/kg p.o</td>
<td>2.40</td>
<td>0.45</td>
<td>1.00</td>
<td>1.20/1.9</td>
</tr>
<tr>
<td>6.</td>
<td>70%EEMI</td>
<td>400mg/kg p.o</td>
<td>2.30</td>
<td>0.60</td>
<td>1.50</td>
<td>1.30/1.20</td>
</tr>
<tr>
<td>9.</td>
<td>AEMI</td>
<td>200 mg/kg p.o</td>
<td>2.40</td>
<td>0.40</td>
<td>1.20</td>
<td>1.35/1.25</td>
</tr>
<tr>
<td>10.</td>
<td>AEMI</td>
<td>400mg/kg p.o</td>
<td>2.45</td>
<td>0.71</td>
<td>1.70</td>
<td>1.40/1.32</td>
</tr>
</tbody>
</table>

DISCUSSION

Clinically, Drugs which are used for the treatment of hypertension, edema, water poisoning and certain kidney diseases are known as Diuretic Drugs or Diuretics. So, Mechanisms of action of this drug is Direct acts on nephron from which tubular lumen and reach the site of action by passing through proximal tubule. A diuretic having some of the activities like natriuretic, chloruretic, Calcitic properties depend on an increase in renal excretion of Na+, Cl-, Ca2+ ion respectively.[14] Example of Loop Diuretic is Furosemide which acts by inhibiting Na+/ Cl2+ ion transport system and main acts in excretion of Na+, K+, Cl- concentration in urine.[14] All given extracts with a polar solvent of different concentration like 60% ethanol and water of Mangifera Indica L. leaves in which presence of alkaloids, saponins, tannins, flavonoids, terpenes and proteins which are mainly responsible for the diuretic activity. The extracts of 60% Ethanolic extracts of Mangifera Indica L. and AEMI
have enhanced the volume of urine significantly at a dose of 200mg/kg and 400mg/kg. Significantly increase excretion of Na+ and K+ in given dose dependence but excretion of Cl−ion not elevated. Also, increase in Na+/K+ ratio shows that given extract having good diuretic Activity. Our results indicate that given extracts of 60% EEMI at 400mg/kg and AEMI 400mg/kg have good diuretic potency. Hence, Lipchitz’s value at the dose of 400 mg/kg of both extracts of 60% EEMI and AEMI given 50% and 65% diuretic activity compared to Furosemide.

CONCLUSION
Leaves of Mangifera Indica L. plant are highly useful ad Diuretic, upon treating with both extracts, i.e. 60% ethanolic extract of Mangifera Indica L. (EEMI) and Aqueous Extract of Mangifera Indica L. (AEMI), gives rise in the volume of urine. This given mechanism and phytochemicals responsible for this activity could not be established from our studies. Hence, our study justifies the claim of leaves of Mangifera Indica L. plant having useful Diuretic potency.

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REFERENCES