Exploring the Diuretic Potential of Ethanolic Extract from Mangifera indica Fruit: A Dose-Related Study.

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Author's Contribution:

H.T and S.A. designed the model and the computational framework and analyzed the data. H.T, S.I and S.A. carried out the implementation. S.J.K. performed the calculations. H.T. and S.I. wrote the manuscript with input from all authors. S.A and A.A conceived the study and were in charge of overall direction and planning.

ABSTRACT

Mangifera indica, commonly called the mango, is a tropical fruit tree that belongs to the *Anacardiaceae* family. Different parts of the plant have been utilized traditionally for various therapeutic purposes. The study intended to explore the diuretic impact of Mangifera indica fruit extracted in ethanol. The ethanol-extracted fruit of Mangifera indica was produced by maceration. The doses of extract selected by going through an extensive literature review are 100 mg/kg, 200 mg/kg, and 400 mg/kg. The diuretic activity was determined by comparing urine volume and

urinary electrolyte excretion. The findings showed that the ethanolic extract of Mangifera indica fruit had a dose-related diuretic effect. The maximum diuretic effect was witnessed at a dose of 400 mg/kg, resulting in a noteworthy improvement in urine and electrolyte output in contrast to the standard control group (furosemide). The extract also showed a significant increase in potassium excretion, suggesting a potassium-sparing diuretic effect. Finally, the ethanolic extract of Mangifera indica fruit has a mild to moderate diuretic effect. Further research is required to explain the mechanisms of action and to identify the responsible active ingredients for the observed activities.

Keywords: *Mangifera Indica*, Furosemide, Diuretic Activity,

1. INTRODUCTION

Mangifera Indica L., or mango tree, is a tropical fruit tree that is widespread in many countries including Pakistan, India, and Bangladesh. It belongs to the *Anacardiaceae* family, which also includes other known plants like cashews and pistachios (1).

One of the most important properties of Mangifera Indica L. is its richness in secondary metabolites. These compounds are plant chemicals that are not essential for the plant's survival but provide various benefits such as repelling predators and disease. Some of the secondary metabolites found in mangoes include flavonoids, tannins, and phenolic compounds known for their antioxidant properties (2).

It is a highly nutritious fruit, containing a high value of vitamins A and C and other essential minerals. However, it should be noted that unripe mangoes fruit are not a rich source of vitamin C as they contain large amounts of tannins that inhibit vitamin C absorption (3).

However, ripe mangoes are known for their stroke-preventing properties, as they are rich in flavonoids and many compounds that have valuable effects on cardiovascular health. The fruit is widely consumed and appreciated for its delicious flavor, bright color, and unique aroma. However, in addition to their culinary uses, mangoes also have several pharmacological effects that can have significant health benefits (4).

Pharmacological effects refer to the various biological effects that a substance can have on the body, including its ability to alter physiological processes and alleviate or prevent disease. Mangoes have multiple bioactive agents, containing phenolic acids, flavonoids, carotenoids, and triterpenoids, which have been shown to exhibit a wide range of pharmacological activities (5).

Among the best-studied pharmacological activities of mango are its anti-inflammatory, antioxidant, antimicrobial, anticancer, and antidiabetic effects. Mango also has neuroprotective properties and may be beneficial in treating various neurological conditions. Given the wide range of potential health benefits, there is growing interest in using mango as a natural therapeutic agent. Ongoing research is investigating the potential of mango and its bioactive components in the avoidance and management of various diseases, and mango may become a valuable addition to the arsenal of natural medicines (6).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The research study employed various chemical agents and solvents, namely absolute ethanol (Merck KGA, Darmstadt, Germany), distilled water, normal saline (Otsuka Pakistan Ltd), and furosemide (Lasix, Sanofi Aventis Pakistan Ltd.). Standard analytical grade chemicals are utilized for performing experiments.

2.2 Origin of plant material

Fresh Mangifera indica fruits collected from the University of Karachi. The plant was verified, and a voucher specimen (MIF-04-17/19) was deposited at the University to be used for future reference and approval purposes.

2.3 Experimental animals

Healthy Wistar rats of both sexes, weighing between 180-260 grams, and aged between 6-8 weeks were selected for the conduction of experiments. These rats were procured from the animal house of the University of Karachi and placed in polypropylene cages. They were allowed to get accustomed to the environment of the laboratory for a week. Each rat was rested in a separate metabolic cage for a day before the commencement of experiments. All rats were supplied with a

standard diet and water as per their requirement. All the animals were handled by internationally recognized guidelines to ensure their welfare (7). The ethical review board of the University of Karachi granted permission for the procedure.

2.4 Synthesis of plant extract

The Mangifera Indica fruits have undergone a process of pounding with tap water to remove impurities followed by hand-cutting into small pieces and then shade drying. The resulting dried fruit was then finely ground into powder and parted into two portions for extraction.

2.5 Synthesis of 80% Ethanol Fruit Extract

The process of obtaining fruit extract involved maceration, where 100 g of desiccated fruit powder was soaked in 400 ml of 80% ethanol for three days. The resulting mixture was then clarified using Whatman class 1 filter paper and treated under reduced pressure using Rotavapor at a high temperature of 40°C to make the extract concentrated. The concentrated extract was subsequently freeze-dried and lyophilized at -20°C, before being stored in a desiccator in a suitable glass container until required.

2.6 Grouping and Dosing of Animals

Five groups of animals were selected each group has five animals. Rats of both sexes were randomly allocated to each group. The first group was administered 2 mL/100 g distilled water orally (the vehicle used for reconstitution of drug) termed as a negative control, the second group was treated with the diuretic reference drug furosemide at 10 mg/kg dose served as a positive control. Groups third – fifth received an ethanolic extract of plant material in doses of 100, 200, and 400 mg/kg. Data obtained from the previous studies are keenly observed to establish all the mentioned doses.

2.7 Determination of Diuretic Activity

The study employed methods previously used in related research to explore the diuretic impact of the Mangifera fruit extract (8). Before the administration of furosemide, water, or test doses of the fruit extract. Animals were set to approach drinking water and restrained to food overnight. Each rat was placed in separate metabolic cages. The sample of urine was taken and examined at specific

intervals for pH and electrolyte levels. The study measured various parameters, including total urine volume and electrolytes concentration (Na+, K+, and Cl-) for each rat. Urinary excretion was calculated by using Eq 1, while the diuretic effect of a given dose of fruit extract was calculated by the ratio of urinary excretion in the test group vs control as presented in Eq 2. Additionally, the comparison between the diuretic effect of extract and furosemide, a standard diuretic drug was done, as described in Eq 3.

1. Urinary Excretion = $\frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100$

2. Diuretic Action = $\frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of the control group}}$

3. Diuretic Activity = $\frac{\text{Diuretic action of test drug}}{\text{The diuretic action of standard drug}}$

2.8 Analytical Procedure

The study examined levels of electrolytes (sodium, potassium, and chloride) in urine samples after administration of ethanol Mangifera fruit extract. An ion-selective electrode analyzer (AVL 9181; Roche, Germany) was used to calculate the concentrations of sodium, potassium, and chloride in a sample of urine. In addition, electrolyte ratios such as Na+: K+ and Cl: K++Na+ were calculated. In addition, the pH of fresh urine samples was determined with a pH meter (WalkLab HP9010 from Trans Instruments, Singapore).

2.9 Statistical Analysis

The data is expressed as mean ± S.E.M, and statistically analyzed by using IBM's SPSS 21.0 software. To measure the differences among more than two groups, ANOVA (one-way analysis of variance) was used, followed by a Dunnet post hoc comparison test. Differences were considered significant at a P-value less than 0.05 ($p< 0.05$).

3. RESULTS

3.1 Analysis of the Diuretic effect of Mangifera Indica ethanolic fruit extracts

3.1.1 Influence on Urine Volume at a dose of 100 mg/kg

Ethanolic fruit extract of Mangifera indica was administered orally at a dose of 100 mg/kg. It was found that rats that received the mentioned dose did not show an increase in diuresis, as evidenced by the low urine output at 4 hours compared to the group of rats that received furosemide. The urine output persisted throughout the study period, resulting in no significant increase in cumulative urinary excretion at 5 and 24 hours after the dose. While the outcome of furosemide, a standard diuretic, was prompt and greater than that of the plant extracts, the 24-hour collective urine output was significantly diverse between the two agents.

3.1.2 Influence on Urine Volume at a dose of 200mg/kg

In this group, it was found that the rats received a 200 mg/kg dose of the ethanolic fruit extract of Mangifera indica fruit, which increased their diuresis, as evidenced by the higher urine output compared to the -ev control rats at the fourth hour later the dose administered. The increase in urine output persisted throughout the study period, resulting in significantly higher cumulative urinary excretion at 5 and 24 hours after the dose compared to the control group. While the effect of furosemide, a standard diuretic, showed a more prompt and greater effect than that of the plant fruit extracts. However, the 24-hour collective urinary excretion was comparable between the negative control and mentioned dose.

3.1.3 Influence on Urine Volume at a dose of 400mg/kg

At hour 5, the highest dose 400 mg/kg showed the maximum effect, with a significant increase starting from the 2nd hour, with a p-value less than 0.001, continuing up to hour 5 (Table 1). Comparing the various doses of the 80% ethanol fruit extract. At 400mg/kg the highest dose of plant extract showed a significant diuresis in comparison to the 100mg/kg and 200mg/kg at various time intervals. The diuretic actions of 200mg/kg and 400 mg/kg were 0.26 and 0.41 respectively, with percent urinary excretion of 53.33% and 82.35%, respectively (Table 1). Furthermore, the % urinary excretion of 400mg/kg was more than the negative control, which was 78.7%.

Groups	The volume of urine (mL) at different time points						%UE	Diuretic action	Diuretic activity
Time (hour)	1	2	3	4	5	24			
DW 2mL/100g	0	0.3	0.4	0.48	0.56	6	78.7	0.39	
F10 mg/kg	5.16	5	5.2	5.1	6	9	200	2.8	
MIEE 100mg/kg	0	0.1	0.2	0.2	0.2	2	40	0.2	0.07
MIEE 200mg/kg	0.3	0.36	0.4	0.4	0.45	4	53.33	0.265	0.09
MIEE 400mg/kg	0.49	0.68	1.79	2	2.2	6	82.35	0.41	0.14

3.2 Effects on Urine electrolytes Concentration

Urine Electrolytes (K+, Cl-, and Na+) were analyzed in samples over a period of 5 hours. There was a significant difference between MIEE100 and MIEE200 and the negative control in measured electrolyte loss. However, MIEE400 caused a significant loss of K+ (level of significance less than 0.001) and Cl- (level of significance less than 0.05), with less effect on Na+ excretion. However, furosemide showed a considerable excretion of all three ions in comparison to the negative control. The Saluretic indices of furosemide for Na+ are 2.5 and Cl- is 2.09 which were greater than the ethanol extracted doses of the fruit of Mangifera indica, while the K+ index of MIEE400 (3.28) and MIEE200 (2 .97) was significantly greater than furosemide (2.31). Also, the Na+: K+ ratios of MIEE100 (0.75), MIEE200 (0.61), and MIEE 400 (0.68) were lesser than furosemide. Higher doses of ethanol fruit extract had comparatively similar effects on the excretion of urine electrolytes. It was also analyzed that chloride excretion of MIEE100, MIEE200, and MIEE400 mg/kg presented a significant difference when compared with control *($p<0.05$).*

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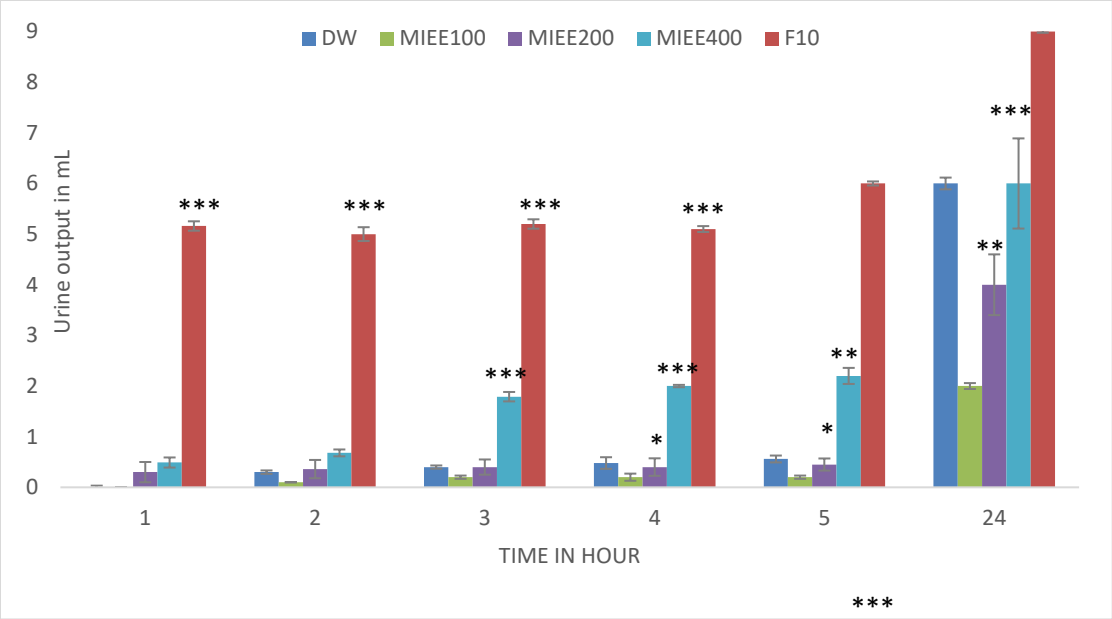

Figure 1: Acute diuretic effect of Mangifera indica ethanol fruit extract (MIEE) at the doses of 100 mg/kg, 200 mg/kg, 400 mg/kg, and furosemide (10 mg/kg). Urine output was calculated at 1, 2, 3, 4, 5, and 24 hours after administration of drugs. Values represent mean ± S.E.M of 3 independent experiments and animals (n= 5). Asterisks indicate significant difference (*$p < 0.05$, **$p < 0.01$***$p < 0.001$). One-way analysis of variance (ANOVA) followed by Dunnet post hoc test performed.

3. DISCUSSION

Diuretics are substances or drugs that are used to increase urine production and facilitate the removal of excess amounts of water and electrolytes from the body. Diuretics are commonly used in the treatment of hypertension, cardiac arrest, and edema (9). This study investigated the diuretic role of ethanolic extracts of Mangifera fruits orally administered in normal rats. The extract pharmacological response at three different doses was compared with the furosemide at a dose of 10 mg/kg, which is the most prescribed diuretic agent in clinical practice (10). Three doses of plant fruit extract were selected for the study. The oral route opted for the administration of drugs because this is the way the population consumes these plant extracts in conventional therapy. The diuretic effect was further confirmed by checking the effect on electrolyte balance. The probable mechanism of action of the diuretic was also evaluated by linking the effect with furosemide, an acute loop diuretic (11). There are two components of diuresis: an increase in the volume of urine (excretion of water) and an increase in the rate of net loss of electrolytes in the urine. The diuretic

components are achieved because of restraining the reabsorption of salt and water into the bloodstream by renal tubules. The diuretic effect of reference drug furosemide attained by increases the excretion of water and sodium by preventing the Na+/K+/2Cl symporter (cotransporter system) in the thick ascending loop of Henley (11, 12), while thiazide diuretics prevent Na+/Cl- (cotransporter system) in the distal convoluted tubule, increase the excretion of Na+ and Cl- by competing for the Cl- binding site (13). The current study showed that at the dose of 400 mg/kg, ethanolic extract of Mangifera indica the rats showed a significant increase in volume of urine in 3 hours, while rats given a dose of 100 mg/kg and 200 mg/kg took 4 hours to increase excretion of urine. In contrast, a single dose of furosemide made a rapid and significant increase in urine volume within 1 hour of drug administration (Fig. 1). The variance in the onset of the diuretic effect of the extract and the control group may be correlated to the absorption of active substances in the gastrointestinal tract and the amount of the active substance responsible for causing the diuretic effect in smaller doses, the number of diuretic components is less, and thus the isolation of the active substances from the fruit extract can reduce the onset of the effect and give more pronounced diuretic effect even at a low dose (14).

Stimulation of diuresis with appropriate doses of all extracts was continued for at least 24 hours (Fig. 1). High-dose extracts resulted in a significant increase in urinary excretion of K+ (228.21% compared to controls) and to a lesser extent Na+ (116.32%). Furosemide increased excretion of K+ (176.57% of controls) and Na+ (150.60%). Hydrochlorothiazide has been reported to increase urinary Na+ and K+ excretion by 50–60% after a single oral dose in healthy rats compared to control. In this regard, ethanolic plant extracts appear to contain multiple active compounds, at least one of which has a thiazide-like diuretic mechanism of action (15).

In summary, current research supports the ethnomedicinal use of Mangifera indica fruits for its diuretic effects. The ethanolic extract at a dose of 400 mg/kg appears to have significant diuretic effects in rats. Although the active substances have not been identified. However, based on the pattern of excretion of water, sodium, and potassium, it appears that at least two types of active ingredients, one with furosemide activity and the other with thiazide activity, are present in these extracts. These results suggest that further investigation and isolation of the active ingredient from the ethanolic fruit extract may be beneficial and provide deep insight into the diuresis mechanisms of Mangifera fruits used in the traditional medicine of the Asian population.

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