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Evaluation of *In-Vitro* and *In-Vivo* Diuretic Activity of Leaves of *Melia azedarach* Linn. in Albino Wistar Rats

Kanimozhi R[1*], Sakthi Abirami M[2]

[1] Department of Pharmacology, Student of pharmacy, College of pharmacy, Madras Medical College, Affiliated to the Tamil Nadu Dr. M.G.R Medical university, Chennai, India.

[2] Department of Pharmacology, Faculty of pharmacy, College of pharmacy, Madras Medical College, Affiliated to the Tamil Nadu Dr. M.G.R Medical university, Chennai, India

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ABSTRACT

Objective: This study set out to assess the diuretic properties of *Melia azedarach* Linn. leaves in albino wistar rats both in vitro and in vivo. **Methods:** Traditionally, Melia azedarach Linn. (Family: Meliaceae) has been used to treat bacterial infections, wound healing, and specific kidney conditions. Phenolic components, tannins, and flavonoids were found in the Ethanolic Extract of *Melia azedarach* Linn. leaves (EEMA), according to the preliminary phytochemical study. The extract's diuretic action was assessed both in vitro and in vivo. EEMA's in vitro diuretic action was assessed using the carbonic anhydrase inhibition assay. The Lipschitz method model was used to assess the in vivo diuretic activity in Albino wistar rats (Eiether sex). Vehicle, standard, and two distinct doses of test medicines were administered to the animals in groups I, II, III, and IV, respectively. Animals were placed in distinct metabolic cages for urine collection as soon as the dosage was completed. A urine sample was taken after five hours, and its diuretic action was examined. **Results:** The presence of several substances with known medicinal properties, such as alkaloids, glycosides, saponins, phenolic compounds, tannins, and flavonoids, was discovered using phytochemical screening. The ethanolic extract of *Melia azedarach* Linn had notable concentrations of primary phytochemicals. The inhibitory potential of carbonic anhydrase was validated by in vitro investigations. Studies conducted in vivo showed that *Melia azedarach* Linn. is safe and that it has diuretic effects on animals, which include increased urine volume and urine alkalinization with electrolyte excretion.

KEYWORDS: Bakain, botanical information, reports on phytochemistry, and pharmacological research.

1. INTRODUCTION

Diuretics are defined as any substance which is responsible for increasing the rate of urine flow and salt (Na^+, K^+ &CL^-) Excretion and to maintain the volume and composition of body fluids in various clinical condition.(1) Diuretics increase the urine output and thus removes the additional extra and intracellular fluid/ water. Any drug that increases the rate of urine flow and salt (Na+, K+, and CL-) excretion as well as maintains the volume and composition of bodily fluids in a variety of clinical conditions is referred to as a diuretic.(1) Diuretics stimulate urine production, which eliminates excess water and intracellular fluid from edematous tissue.

There are two ways that a medication can produce its diuretic effect. That is, they affect the nephron directly and indirectly by changing the filtrate's composition. Increasing the glomerular filtration rate (GFR) has a direct effect on the nephron, while lowering the tubules' re-absorption of fluid and salt has an indirect effect.(2) In India, diuretics were used to treat cardiovascular disease in over 45.5% of patients. Acute pulmonary edema, cerebral edema, congestive heart failure, hypertension, liver cirrhosis, renal failure, nephritic syndrome, and frequently pregnant toxemia are among the many life-threatening conditions for which drug induced diuresis id extremely helpful.

The traditional medications that are now on the market are also linked to a number of negative side effects.

For example;

- Electrolyte problems caused by loop and thiazide diuretics include acute hypovolemia, acid-base imbalance, hypokalemia, hyperuricemia, and hyponatremia.

- Natural diuretics, such as caffeine and alcohol, work by preventing the reabsorption of sodium and the release of the anti-diuretic hormone vasopressin. However, they might have negative side effects, such as weakness, exhaustion, and impotence.[3]

Thus, the search for a novel diuretic that has no negative side effects and maintains the same therapeutic efficacy is still ongoing. Since the beginning of time, naturally occurring medicinal plants have been utilized extensively to cure a wide range of human ailments.

2. MATERIALS AND METHODS

2.1. PROCUREMENT OF *Melia azedarach*

Melia azedarach Linn. leaves were gathered from Arakkonam, Chennai, and verified by Dr. K.N. Sunil Kumar, the Head of the Department of Pharmacognosy and Research Officer at Siddha Central Research Institute, Arumbakkam, Chennai-600106.

PREPARATION OF ETHANOL EXTRACTION

PREPARATION OF EXTRACT: The first stage in phytochemical investigations is extraction. Since ethanol is a universal solvent, it was chosen to extract the active ingredients from the plants independently. The Soxhlet extraction procedure is the extraction technique used.

SOXHLET APPARATUS: At 60–70°C, 250g of Melia Azedarach Linn. extracts that have been coarsely ground are sent through a mesh screen and extracted using ethanol. To concentrate the leaf extract, a rotary vacuum evaporator was used. After noting each extract's colour, consistency, and percentage yield, they were subjected to more thorough phytochemical and pharmacological evaluation.

2.2 PHYTOCHEMICAL ANALYSIS

Methodology for chemical analysis

Test for Alkaloids

a) Mayer's Test: A solution of potassium mercuric iodide (Mayer's reagent) was added to 0.5 ml of the sample. Alkaloids are present when the precipitate is cream-colored (dull white).

b) Dragendroff's Test: A 1 ml solution of potassium bismuth iodide, also known as Dragendroff's reagent, was applied to 1 ml of the sample. Alkaloids are present when there is an orange-red precipitate.

c) Hager's Test: 0.5 ml of Hager's reagent (saturated solution of picric acid) was added to 1 ml of the sample. The presence of alkaloids is indicated by a yellow precipitate.

d) Wagner's test: Two ml of the extract were mixed with a few drops of Wagner's reagent, which is an iodine solution in potassium iodide. When alkaloids are present, a reddish-brown precipitate forms.

Test for Carbohydrate

a) Molisch's Test: After adding a few drops of alcoholic alpha-napthol to the 0.5 ml of sample and gradually adding 0.2 ml of concentrated sulfuric acid through the test tube's walls, a purple to violet color ring formed at the intersection.

b) Benedict' Test: A reddish brown precipitate appears if reducing sugars are present. Benedict's reagent, an alkaline solution containing cupric citrate complex, was added to 1 ml of the sample and heated on a water bath.

Test for Glycosides

After hydrolyzing the extract with mineral acid, the glycone and aglycone moieties were examined.

Legal's test: After adding an alkaline sodium nitroprusside solution and a few drops of pyridine to 2 ml of extract, the extract turned blood red, indicating the presence of glycosides.

Test for Cardiac Glycosides

Keller Killani Test [Test for Deoxy sugars]: A trace amount of ferric chloride and two to three drops of concentrated sulfuric acid were added to two ml of extract after glacial acetic acid. Heart glycosides are present when a reddish brown color appears where two liquids converge.

Test for Saponin

a) Foam froth Test: After treating 1 ml of the sample with 10 ml of water and boiling it for a few minutes, it was filtered. After giving the filtrate a good shake, the stable foam was observed.

b) Froth test: A sodium bicarbonate solution was mixed with five ml of the test sample. After giving the mixture a good shake, let it sit for three minutes. A foam formed that resembled a honeycomb. It shows that saponins are present.

Test for Sterols

Salkowski Test: After adding roughly 0.3 ml of chloroform and a few drops of concentrated sulfuric acid to 0.5 ml of the sample, shaking thoroughly, and letting it stand for a while, the lower layer turned red, signifying the presence of steroids, and the lower layer turned yellow, signifying the presence of triterpenoids.

Test for phenolic compounds

a) Ferric chloride Test: After adding 1 ml of water to 1 ml of the sample, it simmered for a few minutes before being filtered. A bluish-black color was generated after the filtrate was treated with a ferric chloride solution.

b) Lead acetate test: One ml of the sample was diluted with five ml of distilled water, and a few drops of a 1% lead acetate aqueous solution were added. The presence of phenols is indicated by the formation of a yellow precipitate.

Test for Tannins

a) Lead acetate Test: Five ml of extract were mixed with a few drops of lead acetate. Tanning agents are present when a yellow or red precipitate forms.

b) Gelatin Test: 10% sodium chloride and 1% gelatin were added to the extracts. The presence of tannins is shown by the production of white precipitate.

Test for Flavonoids

a) Shinoda Test (Magnesium Hydrochloride reduction Test): A few pieces of magnesium ribbon were added to 1 ml of the sample, followed by a few drops of strong hydrochloric acid. The presence of flavonoids results in a magenta color.

Test for Proteins and Amino acids

a) Millon's Test: Two ml of Millon's reagent (mercuric nitrate in nitric acid with traces of nitrous acid) were applied to one ml of the sample. A white precipitate formed, which became crimson when heated gently.

b) Ninhydrin Test: After adding 0.5 ml of a 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate) to 1 ml of the sample and boiling it in a water bath, the violet coloration that results shows the presence of proteins and amino acids.

Tests for fats and fixed oils

a) Stain Test: A tiny amount of the sample was sandwiched between two filter sheets; the presence of fixed oils is indicated by the stain on one of the sheets.

PHARMACOLOGICAL STUDIES

2.3 IN-VITRO STUDIES

In-vitro Study for Diuretic Activity

Carbonic anhydrase inhibition assay (S.I. Ibrahim, et al 2016) [4]

Acetazolamide was one of the first synthetic non-mercurial diuretics. The mode of action was found to be inhibition of carbonic anhydrase. Carbonic anhydrase is a zinc- containing enzyme that catalyzes the reversible hydration (or hydroxylation) of CO_2 to form H_2CO_3 which dissociates non-enzymatically into HCO_3^- and H^+.

Procedure

The spectrophotometric test was carried out at 25°C in a pH 7.4 (20 mM) HEPES (Hydroxyethylpiperazine Ethane Sulfonic Acid)-Tris buffer. Each inhibitory tube contained 400 µL of carbonic anhydrase enzyme solution (0.1 mg/mL HEPES-Tris buffer), 1400 µL of HEPES-Tris buffer solution, and 400 µL of Melia azedarach (EEMA) ethanolic extract in HPLC-grade Dimethyl Sulfoxide (keep 10% of the final concentration). For fifteen minutes, the mixed solution was pre-incubated at 25°C. 400 µL of HPLC-grade methanol was used to prepare the substrate, p-nitro phenyl acetate (p-NPA) (0.7 mM), and to initiate the reaction.

In a UV spectrophotometer made by Elico (India), the amount of product produced was measured at 400 nm. It was assumed that the controlled chemical had 100% activity. Each concentration was utilized in triplicate for all tests, and the mean of the triplicate was used to reflect the results. Acetazolamide was used as a standard.

2.4 IN-VIVO STUDIES

Experimental Animals

The Institutional Animal Ethics Committee granted approval for the current study, and the procedure complied with national CPCSEA/IAEC rules (approved number 03/AEL/IAEC/MMC, dated 14.08.2024). The study's albino wistar rats were purchased from Animal House, Madras Medical College in Chennai, India.

Selection of Animal Species

Albino wistar rats of either sex were the favored rodent species, while other rodent species might be employed. It was usual practice to use laboratory strains of healthy young adults. Women ought to be non-pregnant and nulliparous. Each animal should be between 6 and 8 weeks old when dosing begins, and its weight (150–200g) should be within ±20% of the mean weight of any animals that have already received a dosage. Madras Medical College at Chennai's Animal Experiments Laboratory provided sexually mature albino wistar rats of both sexes. Every animal was housed in a regular environment with a temperature of 22±3°C. The animals were fed a regular pellet diet and had unrestricted access to water.

Housing and Feeding Conditions

The animal experiment room should have a temperature of 22±3°C. Aim for 50–60% relative humidity, albeit it should be at least 30% and ideally not higher than 70%, unless the space is being cleaned. Artificial lighting with a 12-hour light-dark cycle should be used. Conventional laboratory meals and an unrestricted supply of drinking water can be employed for feeding. Animals may be housed in dose-specific groups, but the quantity of animals in each cage shouldn't make it difficult to observe each one clearly.

Animal Identification

All animal cages used in the study had a proper identification i.e., labels. Each animal in the cage was marked on tail with picric acid for their appropriate identification.

Preparation of Animals

The animals were chosen at random, labeled to enable unique identification, and housed in their cages for a minimum of seven days before the dosage to give them time to adjust to the lab environment. Before the Melia azedarach was administered, rats were denied food for the whole night (but not water for 16–18 hours).

Test substance*: Melia azedarach*

Animal source: Animal Experimental Laboratory, MMC, Chennai-03.

Animals: Albino wistar rats (Either sex)

Age: 6-8 weeks Body weight on day 0: 150-200g

Acclimatization: Seven days prior to dosing

Veterinary examination: Prior and at the end of the acclimatization period

Identification of animals: By cage number, animal number and individual marking by using picric acid.

Route of administration: Oral

Water: Aqua guard portable water in polypropylene bottles.

Housing & Environment: The animals were housed in polypropylene cages provided with bedding of husk.

Housing temperature: 22°C ±3°C.

Relative humidity: 30% to 70%.

Air changes: 10 to 15 per hour

Dark and light cycle: 12:12 hours

Duration: 1 month.

ACUTE TOXICITY STUDY

Prashant Kumar et al. (2015) used albino wistar rats (150-200g) to conduct an acute oral toxicity investigation of Melia azedarach Linn. No more than 2000 mg/kg of the plant is harmful. Accordingly, 200 mg/kg (1/10th of 2000 mg/kg) and 400 mg/kg (1/5th of 2000 mg/kg) were administered as treatment doses in accordance with the OECD's fixed-dose technique.[5][6]

EVALUATION OF DIURETIC ACTIVITY (S.H. Bhusan et al. 2012)

Method: Diuretic activity in rats by LIPSCHITZ method. [7][8][9]

Procedure

The study began with the animals fasting for eight hours, but they were given unlimited access to drinking water. Group I, II, III, and IV animals received standard, vehicle, and two distinct dosages of test medicines, respectively. Additionally, oral gavage of 5 ml of 0.9% normal saline per 100 g body weight was administered. The animals were immediately placed in different metabolic cages to collect urine following medication. Five hours later, a urine sample was taken and its diuretic activity was examined.

Table 1: Grouping of animals

GROUP NO	GROUP NAME	TREATMENT AND ADMINISTRATION	NO. OF ANIMALS
GROUP-I	Vehicle control	Distilled water 2ml/100g, p.o	6
GROUP-II	Standard control	Furosemide 10mg/kg, p.o.	6
GROUP-III	Test group-I	200mg/kg of *Melia azedarach* Linn. p.o	6
GROUP-IV	Test group-II	400mg/kg of *Melia azedarach* Linn. p.o	6

EVALUATION PARAMETERS

Urine volumes

Urine samples were taken from each group of drug-treated animals after they had been in a metabolic cage for five hours. Then, using a calibrated measuring cylinder, the collected urine samples were measured individually.

Urinary pH

A glass electrode pH meter was used to measure each animal's urine sample independently in order to estimate the pH of the urine.

Estimation of urinary electrolyte

Using the Ion Selective Electrode technique, urine samples from animals were examined for the amounts of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions, respectively.

Estimation of urine osmolarity

The Vapour Pressure Osmometry method was used to evaluate the osmolarity of urine samples. [1-2].

STATSTICAL ANALYSIS

The mean ± standard error of mean (SEM) was used to express the results. Using Graph Pad Prism, version 10.2.2, the one-way analysis of variance test (ANOVA) was used to examine all data. Dunnett's multiple comparison test was then performed, with the significance threshold set at $P < 0.05$, $P < 0.01$ and $P < 0.001$ vs control.[1-2]

3. RESULTS AND DISCUSSION

3.1. PERCENTAGE YIELD

The Soxhlet extraction method was used to extract Melia azedarach Linn. using an alcoholic solvent.

The percentage yield was illustrated in the given **Table no.2.**

Table no. 2: Percentage yield of Melia azedarach extraction

S.NO	SOLVENTS USED	PERCENTAGE YIELD (% W/W)
1.	Ethanol	38.27

3.2. ORGANOLEPTIC CHARACTERS

The *Melia azedarach* Linn. was subjected to various organoleptic tests and the results were determined and illustrated in **Table no. 3**

Table-3: Organoleptic characters of Melia azedarach

S.NO	CHARACTER	OBSERVATION
1.	Colour	Dark green
2.	Odour	Pungent
3.	Texture	Fine powder

Melia azedarach was evaluated for organoleptic characters and the extract was found to be fine powder in appearance and dark green in colour. Its odour were found to be pungent.

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3.3. PHYTOCHEMICAL ANALYSIS

The Phytochemicals study was performed on extract of *Melia azedarach* Linn. and the observed results were depicted in the **Table no.4.**

Table no.4: Phytochemical analysis of *Melia azedarach*

S.NO	PHYTOCHEMICAL	TEST	RESULTS
1.	Alkaloids	Mayer's Test	+
		Dragendroff's Test	+
		Wagner's Test	+
		Hager's Test	+
2.	Carbohydrates	Molisch's Test	+
		Benedict's Test	+
3.	Glycoside	Legal's Test	+
		Keller Killiani Test	+
4.	Saponin	Froth Test	+
		Foam Test	+
5.	Steroids	Salkowski's Test	+
6.	Phenols	Ferric Chloride Test	+
7.	Tannins	Gelatin Test	+
8.	Flavonoids	Shinoda test	+
9.	Fats & Fixed Oils	Stain test	_
10.	Proteins & Amino acid	Millon's test	+
		Ninhydrin test	_

(+) indicates presence of active constituents, (-) indicates Absence of active constituents

According to the findings, the extract of Melia azedarach contained phytochemicals including alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, and saponins, but it lacked proteins, amino acids, lipids, and fixed oils.

3.4. *IN-VITRO* STUDIES

3.4.1. Carbonic anhydrase inhibition assay

The carbonic anhydrase inhibition activity of the test drug *Melia azedarach* and standard drug, acetazolamide was found to be **54.05 µg/ml** and **19.07µg/ml** respectively.

Table 5: Percentage (%) Inhibition of standard drug- Acetazolamide

CONCENTRATION (µg)	ABSORBANCE			%INHIBITION (MEAN± SD)
	SINGLET	DOUBLET	TRIPLET	
10	0.438	0.431	0.444	38.759 ± 0.91
20	0.366	0.362	0.367	48.927 ± 0.37
40	0.275	0.27	0.279	61.567 ± 0.63
80	0.159	0.152	0.156	78.218 ± 0.49
160	0.092	0.094	0.098	86.753 ± 0.42
320	0.064	0.061	0.067	91.044 ± 0.41

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Table 6: Percentage (%) Inhibition of test drug- *Melia azedarach* Linn.

CONCENTRATION (µg)	ABSORBANCE			%INHIBITION (MEAN± SD)
	SINGLET	DOUBLET	TRIPLET	
10	0.632	0.637	0.639	11.007 ± 0.50
20	0.54	0.548	0.542	23.973 ± 0.58
40	0.399	0.395	0.393	44.636 ± 0.42
80	0.266	0.261	0.267	62.966 ± 0.44
160	0.163	0.165	0.169	76.819 ± 0.42
320	0.091	0.097	0.094	86.847 ± 0.41

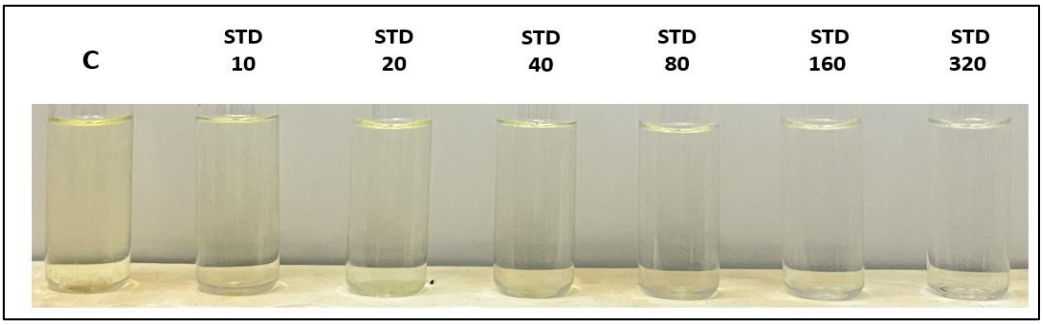

Figure 1: Test tubes with various concentration of standard and test drug

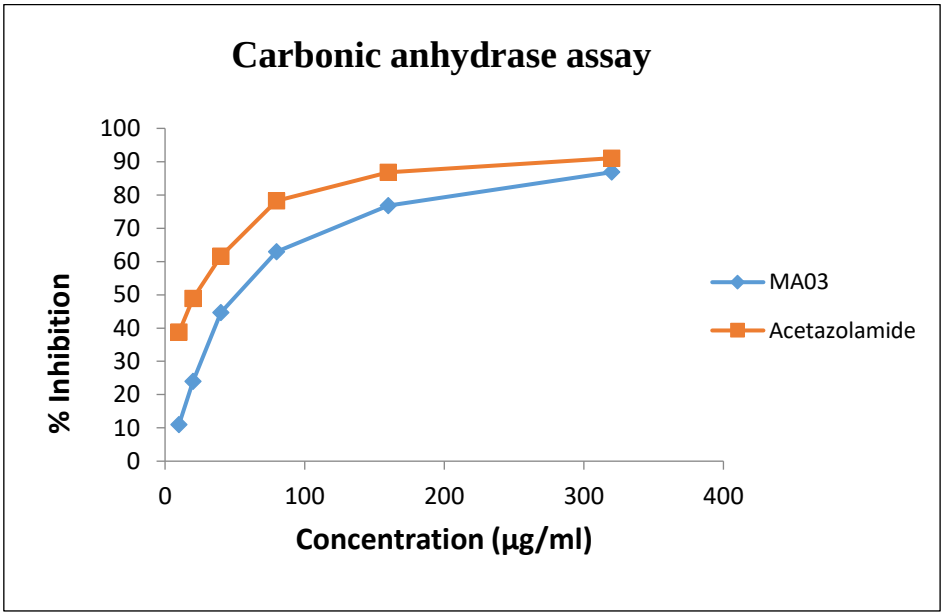

Figure 2: Carbonic anhydrase inhibitory assay – graphical representation

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3.5. *IN-VIVO* STUDIES

3.5.1. DIURETIC ACTIVITY

3.5.1.1. Urine volume

Effect of *Melia azedarach* on urine volume was assessed and the results were depicted in **table 7** and **figure 3**.

Table 7: Effect of *Melia* azedarach on urine volume

S.NO	GROUP	URINE VOLUME (ml)
1	Normal saline	5.6±0.02
2	Standard (Furosemide)	8.8±0.03****
3	Low dose of *Melia azedarach* (200mg/kg)	7.8±0.05****
4	High dose of *Melia azedarach* (400mg/kg)	8.3±0.03****

All values are expressed as Mean ± SEM, (n=6), **** P<0.0001, when compared to normal control group.

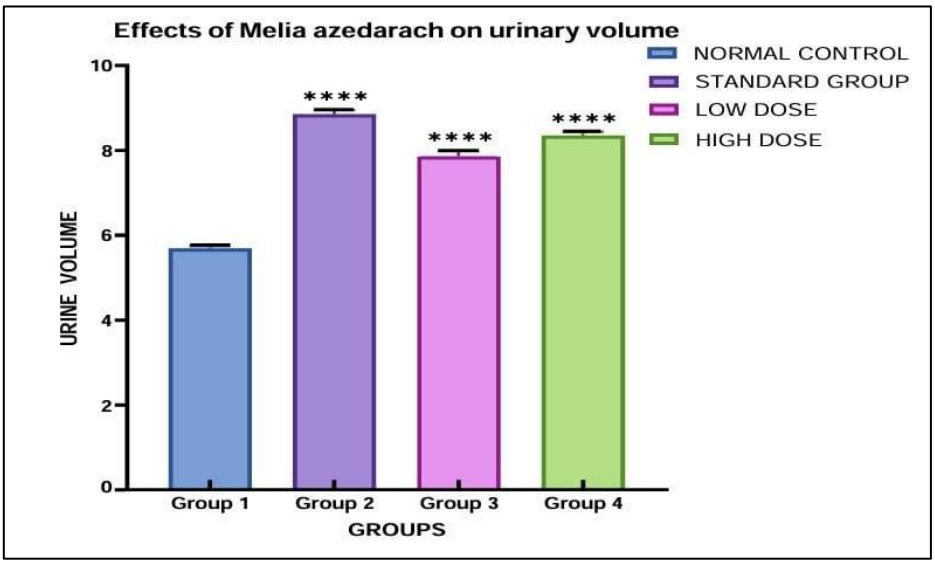

Figure 3: Effect of *Melia azedarach* on urine volume.

All values are expressed as Mean ± SEM, (n=6), **** P< 0.0001, when compared to normal control group.

From the data showed above, there was a significant increase (P<0.0001) in urine volume of standard and test drug treated group when compared with normal control group. Urine volume of the standard drug was found to be **8.8 ± 0.03**. This score showed that there was significant increase (P < 0.0001) in urine volume. At the lower dose (200mg/kg), the urine volume was found to be **7.8 ± 0.05**. It also shows that there was significant increase (P < 0.0001) in urine volume when compared to normal control group. At the higher dose (400mg/kg), urine volume was found to be **8.3 ± 0.03** and shows significant increase (P < 0.0001) in urine volume when compared to normal control group. Among the low dose and high dose treated groups, both the groups showed increased urine output which was nearly equivalent to standard drug treated group.

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3.5.1.2 Urinary pH

Effect of *Melia azedarach* on urinary pH was determined and the results were given in the **table 8** and **figure 4**.

Table 8: Effect of *Melia azedarach* on urinary pH

S.NO	GROUP	URINARY pH
1	Normal saline	7.3±0.01
2	Standard (Furosemide)	8.6±0.03****
3	Low dose of *Melia azedarach* (200mg/kg)	7.8±0.01****
4	High dose of *Melia azedarach* (400mg/kg)	8.1±0.04****

All values are expressed as Mean ± SEM, (n=6), **** P< 0.0001, when compared to normal control group.

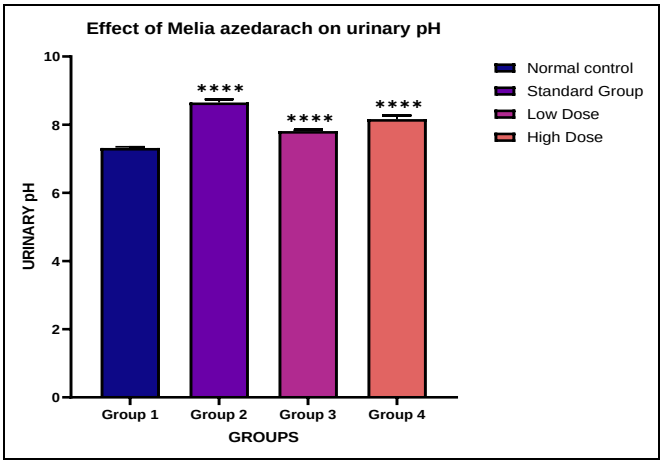

Figure 4: Effect of *Melia azedarach* on urinary pH

All values are expressed as Mean ± SEM, (n=6), **** P< 0.0001, when compared to normal control group.

The results stated above confirms that, there was a significant increase (P<0.0001) in urinary pH of standard and test drug treated group when compared with normal control group. Urinary pH of the standard drug was found to be **8.6 ± 0.03**. This score showed that there was significant increase (P < 0.0001) in urinary pH. At the lower dose (200mg/kg), the urinary pH was found to be **7.8 ± 0.01**. It also shows that there was significant increase (P < 0.0001) in urine volume when compared to normal control group. At the higher dose (400mg/kg), urinary pH was found to be **8.1± 0.04** and shows significant increase (P < 0.0001) in urinary pH when compared to normal control group. Among the low dose and high dose treated groups, the higher dose treated group shows increased urinary pH which was nearly equivalent to standard drug treated group.

3.5.1.3. Estimation of urinary electrolyte

Effect of *Melia azedarach* on urinary sodium (Na^+) was assessed and the results were depicted in the **table 9** and **figure 5, 6 and 7**.

Table 9: Effect of *Melia azedarach* on urinary electrolyte

S. No	GROUP	SODIUM (Na+) (mEq/L)	POTASSIUM (K+) (mEq/L)	CHLORIDE (Cl-) (mEq/L)
1	Normal saline	92.78±1.03	72±0.69	125.61±0.55
2	Standard (Furosemide)	118.91±1.27****	87.28±0.95****	158.60±1.02****
3	Low dose of *Melia azedarach* (200mg/kg)	109.88±0.72****	77.32±0.23***	148.97±1.00****
4	High dose of *Melia azedarach* (400mg/kg)	112.58±0.77****	88.73±0.89****	157.96±0.75****

All values are expressed as Mean ± SEM, (n=6), **** P < 0.0001, *** P < 0.001, when compared to normal control group.

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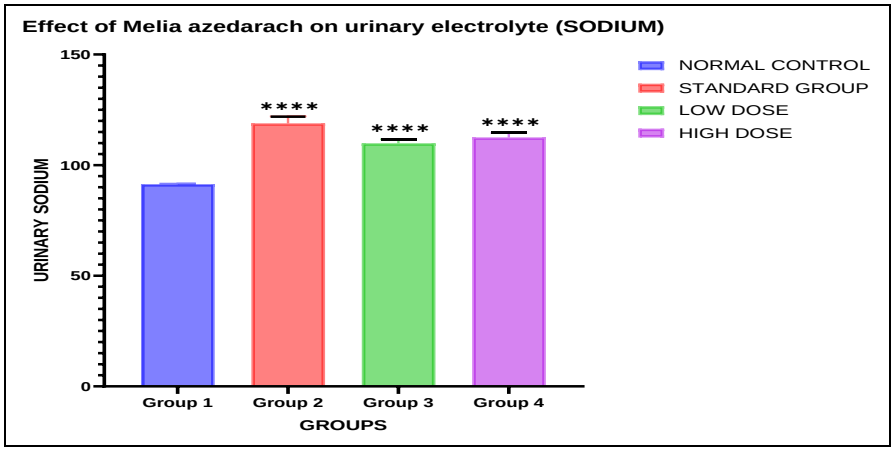

Figure 5: Effect of *Melia azedarach* on urinary electrolyte

All values are expressed as Mean ± SEM, (n=6), **** P < 0.0001, when compared to normal control group.

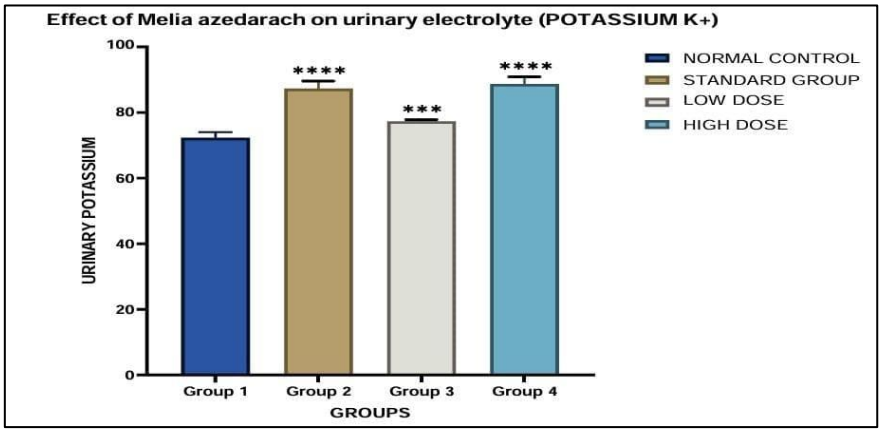

Figure 6: Effect of *Melia azedarach* on urinary electrolyte

All values are expressed as Mean ± SEM, (n=6), **** P < 0.0001, *** P < 0.001, when compared to normal control group.

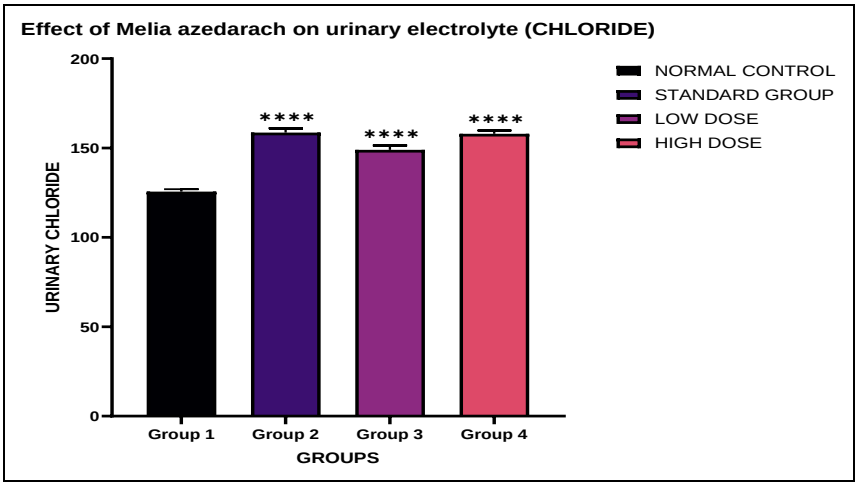

Figure 7: Effect of *Melia azedarach* on urinary electrolyte

All values are expressed as Mean ± SEM, (n=6), **** P < 0.0001, when compared to normal control group.

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The results confirm that, there was significant increase in urinary electrolyte excretion of standard and test drug treated group when compared with normal control group. Urinary electrolyte excretion of the standard drug was found to be **118.91±1.27mEq/L** with significance of ($P < 0.0001$) for sodium, **87.28±0.95 mEq/L** with significance of ($P < 0.001$) for potassium and **158.60±1.02 mEq/L** with significance of ($P < 0.0001$) for chloride ion excretion, when compared to normal control group. At the lower dose (200mg/kg), the urinary electrolyte excretion was found to be **109.88±0.72 mEq/L** with significance of ($P < 0.001$) for sodium, **77.32±0.23 mEq/L** with significance of ($P < 0.01$) for potassium and **148.97±1.00 mEq/L** with significance of ($P < 0.001$) for chloride ion respectively, when compared to normal control group. At the higher dose (400mg/kg), electrolyte excretion was found to be **112.58±0.77mEq/L** with significance of ($P < 0.0001$) for sodium, **88.73±0.89 mEq/L** with significance of ($P < 0.01$) for potassium and **157.96±0.75 mEq/L** with significance of ($P < 0.0001$) for chloride ion respectively, when compared to normal control group. Among the two different doses of the test drug, a group treated with higher dose (400mg/kg) shows the increased electrolyte excretion which was nearly equivalent to standard drug treated group.

3.5.1.4. Estimation of urinary osmolarity

Effect of *Melia azedarach* on urinary osmolarity was determined and the results were depicted in **table 10** and **figure 8**.

Table 10: Effect of *Melia azedarach* on urinary osmolarity

S.NO	GROUP	URINARY OSMOLARITY (mOsm/kg)
1	Normal saline	291±0.2
2	Standard (Furosemide)	308±0.09****
3	Low dose of *Melia azedarach* (200mg/kg)	297±0.12****
4	High dose of *Melia azedarach* (400mg/kg)	302±0.02****

All values are expressed as Mean ± SEM, (n=6), **** $P < 0.0001$, when compared to normal control group.

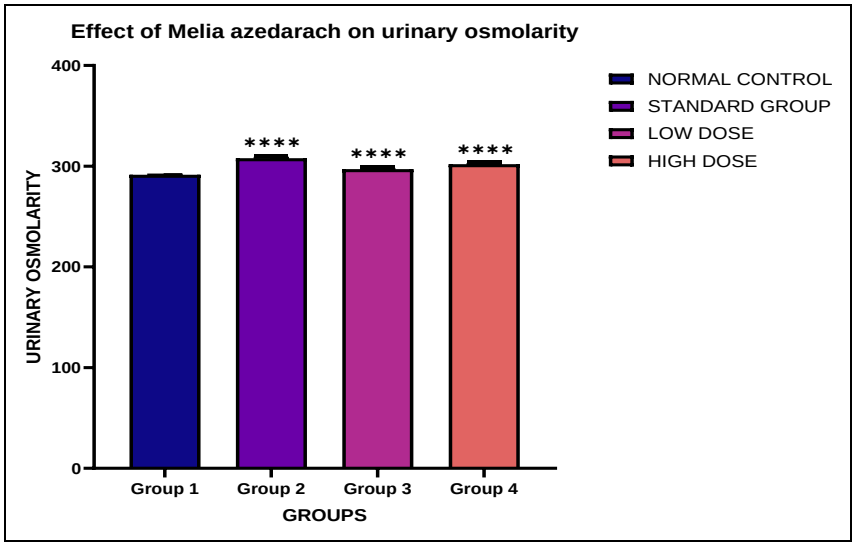

Figure 8: Effect of *Melia azedarach* on urinary osmolarity

All values are expressed as Mean ± SEM, (n=6), **** $P < 0.0001$, when compared to normal control group.

From the data showed above, there was a significant increase ($P<0.0001$) in urine osmolarity of standard and test drug treated group when compared with normal control group. Urine osmolarity of the standard drug was found to be **308 ± 0.09 (mOsm/kg)**. This score showed that there was significant increase ($P < 0.0001$) in urine osmolarity. At the lower dose (200mg/kg), the urine osmolarity was found to be **297 ± 0.12 (mOsm/kg)**. It also shows that there was significant increase ($P < 0.0001$) in urine osmolarity when compared to normal control group. At the higher dose (400mg/kg), osmolarity of the urine was found to be **302 ± 0.02 (mOsm/kg)** and that shows significant increase ($P < 0.0001$) in urine osmolarity when compared to normal control group. Among the low dose and high dose treated groups, the group treated with higher dose showed increased urinary osmolarity which was nearly equivalent to standard drug treated group.

CONCLUSION

The study's findings indicate that Melia azedarach Linn. leaves' ethanolic extract (EEMT) has diuretic properties. Therefore, the current research backs up the plant's long-standing claim to be a diuretic. Melia azedarach's diuretic qualities were evaluated through in vitro research. It demonstrated the test drug's ability to inhibit carbonic anhydrase. The study validated Melia azedarach's in vitro diuretic action, although acetazolamide exhibited more inhibitory activity at a lower dose. The results of the electrolyte and urine PH analyses suggest that the extracts may have several ways of working. Alkaloids, terpenoids, flavonoids, and anthroquinones are the characteristic constituents of M. azedarach, according to phytochemical investigations. For future perspective, it can be further confirmed by molecular investigations of the chemicals responsible for the diuretic activity to study the specific mechanism of action by which the plant possesses the activity.

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