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Evaluation of Diuretic Activity of *Pongamia pinnata* Seed in Albino Wistar Rats

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ABSTRACT

Objective: The aim of this work was to investigate the diuretic effect of *Pongamia pinnata* Ethanolic Seed Extract in Albino Wistar Rats.**Methods:** Phytochemical studies were performed for the detection of phytoconstituents and the *in vivo* diuretic activity was performed by Lipschitz method in Albino Wistar rats. The parameters evaluated in order to demonstrate the diuretic effect are Urine volume, urinary pH, urinary electrolytes, sodium ion concentration, potassium ion concentration and chloride ion concentration. The results were analysed using one-way ANOVA and p values were calculated to find the significance of the results.**Results:** Phytochemical analysis reported the presence of steroids, alkaloids, cardiac glycosides, tannins, flavonoids, phenolic compounds, proteins and amino acids, fats and fixed oils were present. *Invitro* Carbonic anhydrase inhibition assay showed that the *Pongamia pinnata* Ethanolic Seed Extract (PPESE) possessed the IC50 of 130.43 µg/ml. *In vivo* results showed that treatment with PPESE is effective in acting as a Diuretic agent by increasing the urine volume, urinary pH, urinary electrolytes, sodium ion concentration, potassium ion concentration and chloride ion concentration. Treatment with PPESE at the dose of 400 mg/kg increases urine volume to 8.2 ml, urine pH to 8.1, sodium ion concentration to 113 mEq/L, potassium ion concentration to 87.46 mEq/L and Chloride ion concentration to 159.66 mEq/L.

Keywords: Diuretics; *Pongamia pinnata*; Hydrochlorothiazide; *Pongamia pinnata* Ethanolic Seed Extract (PPESE); Lipschitz test; Carbonic Anhydrase Inhibition Assay

1. INTRODUCTION

Plants are the richest source of organic chemicals. (1) The use of medicinal plants for treatment for humans and animals are practiced from time immemorial. (2) Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system. Diuretics are responsible to increase the rate of urine flow, sodium excretion and to maintain the volume and composition of body fluids in various clinical disorders. (3)

The diuretic action of drug can be achieved by two different mechanisms. That they are, direct action on the nephron and indirectly by modifying the content of filtrate. Direct action on the nephron occurs by increasing the glomerular filtration rate (GFR) and indirect action occurs by decreasing the fluid and salt re-absorption from the tubules. (4)

More than 45.5% of patients were on diuretics for the management of cardiovascular disease in India. Drugs that induce diuresis are known as diuretics. Diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, and hypertension (5). At the same time, there are several adverse effects associated with currently available conventional drugs. For example; Loop and thiazide diuretics cause electrolyte abnormalities includes hypokalemia, hypernatremia, acid-base imbalance, metabolic abnormalities and acute hypovolemia. Naturally occurring diuretics include caffeine (in coffee, tea, and cola), which inhibit Na+ reabsorption and alcohol (in beer, wine, and mixed drinks), which inhibit secretion of anti-diuretic hormones. Medicinal plants have been widely used as a source for the treatment of human disorders since the ancient time to this date. (6)

So, the research for new diuretic agent that retains same therapeutic efficacy and devoid of adverse effects are continued. Also, there have been increased waves of interest in the field of research in natural products chemistry.

1.1 Chemistry of plant diuretics (7)

Wide ranges of phytoconstituents were responsible for diuretic activity includes Alkaloids, Glycosides, Tannins, Phenolics, Coumarins, Triterpenoids etc. These phytoconstituents present in plant exert desired pharmacological effect on body and thus act as natural diuretic. Phenolics (flavonoids and tannins) of *Terminalia arjuna, Acacia suma, Camellia sinensis, Cuscuta reflexa, Mimusops elengi;* Alkaloids of *Aerva lanata, Erythrina indica, Cordia rothii, Azima tetracantha*; Coumarins of *Daucus carota;* Triterpenes of *Taraxacum officinale, Abutilon indicum*; Saponins of *Asparagus racemosus, Tribulus terrestris*; Sesquiterpenes lactones of *Taraxacum officinale*; Glycosides of *Opuntia ficus indica, Moringa oleifera* might be involved in the mechanism of diuretic activity.

1.2 Pharmacological activities

Natural Diuretics act by increasing the urine output as well as urinary electrolyte concentration. *Lepidium sativum, Costus speciosus, Phyla nodiflora, Withania coagulans, Tylophora indica, Thespesia populnea, Phyllanthus fraternus, Mimosa pudica* increases the sodium and potassium ion concentration in urine. *Spilanthes acmella, Tribulus alatus* act *as* loop diuretics. *Rungia repens* might cause risk of hypokalaemia due to increase in potassium level in urine. Diuretic agents have very wide application in the treatment of various chronical diseases associated with oedema. They are generally prescribed for the treatment of Hypertension, Congestive heart failure, Glaucoma, Diabetes insipidus and Liver ailments.

India is called the botanical garden of the world for its rich natural resources. Over 6000 plants in India are in used in traditional, folklore and herbal medicine. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used. Plants have a long therapeutic history over thousands of years and still considered to be a promising source of medicine in the traditional health care system. The efficacy and safety of herbal medicines have turned the major pharmaceutical population towards medicinal plant's research.

The Phytochemical evaluation of *Pongamia pinnata* seeds reported the presence of flavonoids, phenolic compounds, alkaloids, saponins, glycosides, proteins and amino acids, fats and fixed oils. So, this plant was subjected for the evaluation of Diuretic activity by *in vitro* and *in vivo* methods.

2. PLANT PROFILE

Table : 1[8]

Botanical name	*Pongamia pinnata* (Linn.) Merr
Synonyms	*Millettia pinnata, Derris indica* (lam), *Pongamia glabra* (vent).
Some species of Pongamia	*Pongamia ovalifolia, Pongamia glabra,* *Pongamia uliginosa, Pongamia velutina* *Pongamia pinnata*
Taxonomy	Kingdom: Plantae Division: Magnoliophyta Class: Magnoliopsida Order: Fabales Family: Fabaceae Genus: Pongamia Species: Pinnata
Vernacular names	Korach, Pungai, Karanj, Indian beach, Hulagilu, Ghrthakaranja, Daharakaranja.
Plant constituents	Alkaloids, Glycosides, Carbohydrates, Sterols, Phenolic compounds, Tannins, Flavonoids, and Saponins, Proteins and amino acids, Fats and fixed oils.
Uses	Antioxidant, Antimicrobial, Antiparasitic, Anti- inflammatory, Anti-convulsant, Anti-diabetic, Anti-hyperammonemic, Cytotoxicity, Anthelminthic, Insecticidal and Immunomodulatory activity.

3. MATERIALS AND METHODS

3.1 PROCUREMENT OF PLANT SEED AND EXTRACTION PROCESS

3.1.1 PROCUREMENT OF *Pongamia pinnata* SEED

Pongamia pinnata was procured from the market place, Chennai and authenticated by Dr. K.N. Sunil Kumar, Research officer, Siddha Central Research Institute, Arumbakkam.

3.1.2 PREPARATION OF EXTRACT OF *Pongamia pinnata* SEED

Soxhlet Extraction of *Pongamia pinnata*

In Soxhlet extraction, Solvent extraction of solid extracts, which is commonly known as solid-liquid extraction (also referred to as leaching or lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid extract pre-treatment. Conventional Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction field.

Procedure

Pongamia pinnata seed should be in fresh or dried. It needs to be crushed and in coarse form. All equipment should be too assembled. And then 50 g extract and 100 ml of ethanol solvent was added to a round bottom flask, which is attached to a Soxhlet apparatus and condenser on heating mantle. The solvent was heated using the heating mantle at 60-80 ℃ and was begun to evaporate, moving through the apparatus to the condenser. The condensate was dripped into the reservoir and if the level of solvent reaches the siphon, it poured back into the flask and then cycle was begun again. Once the extraction sets up, it can be left to run without direct supervision. It wasn't be advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, hence a technician or other lab user should be made aware. It was undergone for 24 hours and then the equipment was turned off.

3.2 PHYTOCHEMICAL ANALYSIS

3.2.1 Methodology for Chemical analysis

Test for Carbohydrate

a) Molisch's Test: To the 0.5ml of sample, few drops of alcoholic alpha naphthol and 0.2ml of concentrated sulfuric acid were added slowly through the sides of the test tube. A purple to violet colour ring at the junction indicates the presence of Carbohydrate.

b) Benedict' Test: To 1ml of sample, few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) were added and boiled on water bath. The formation of a reddish-brown precipitate indicates the presence of Reducing Sugars.

c) Fehling's Test: To 1ml of sample, Fehling's solution A and B were added and heated for few minutes. The presence of Carbohydrates is indicated by the appearance of brick red precipitate.

Test for Proteins and Amino acids

a) Millon's Test: The sample was treated with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). Appearance of a white precipitate which turns red upon gentle heating indicates the presence of Proteins.

b) Biuret Test: The sample was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Formation of a Violet colour is an indication of the presence of Proteins.

c) Xanthoprotein Test: The sample was treated with 2ml of con. nitric acid. Appearance of Orange colour indicates the presence of Proteins.

Test for Alkaloids

a) Mayer's Test: To 1ml of sample, Mayer's reagent [Potassium mercuric iodide solution] was added. Formation of Cream colour precipitate indicates the presence of Alkaloids.

b) Dragendroff's Test: To 1ml of sample, Dragendroff's reagent [Potassium bismuth iodide solution] was added. Formation of a Reddish-brown precipitate is an indication of the presence of Alkaloids.

c) Hager's Test: To 1ml of sample, Hager's reagent [Saturated solution of Picric acid] was added. Appearance of a yellow colour precipitate is an indication of the presence of Alkaloids.

Test for Glycosides

Legal's Test: To 1ml of sample, few drops of pyridine and alkaline sodium nitroprusside solution were added. Appearance of blood red colour indicates the presence of Glycosides.

Test for Cardiac Glycosides

Keller Killiani Test: The Test substance was added with 0.4ml of glacial acetic acid and a little amount of ferric chloride. The mixture was transferred to a small test tube and then 0.5ml of con. sulphuric acid was added. Appearance of blue colour in the acetic layer indicates the presence of Cardiac Glycosides.

Test for Phenolic compounds

Ferric chloride Test: The sample was treated with 1ml of water and boiled for few minutes then it was filtered. The filtrate was treated with ferric chloride solution. Appearance of bluish black colour indicates the presence of Phenolic compounds.

Test for Flavonoids

Alkaline reagent Test: The sample was treated with 1ml of sodium hydroxide. Appearance of yellow colour indicates the presence of Flavonoids.

Test for Saponin

Foam froth Test: The sample was treated with 10ml of water and boiled for few mins then it was filtered. The filtrate was shaken well and noted for the stable froth. A 1 cm layer of foam is an indication of the presence of Saponins.

Test for Tannins

a) Gelatin Test: The sample was treated with 2ml of 1% Gelatin and 10% sodium chloride. Appearance of a white precipitate is an indication of the presence of Tannins.

b) Lead acetate Test: To 2ml of sample, few drops of lead acetate solution were added. Appearance of a white precipitate indicates presence of tannins.

Test for Steroids or sterols

Liebermann Burchard Test: The sample is treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of con. Sulphuric acid. The colour changes from red to bluish green indicate the presence of Sterols.

Test for Terpenoids

Noller's Test: Two or three granules or tin metal were dissolved in 2ml thionyl chloride solution and added to 1ml of the extract and warmed. The formation of a pink colour is an indication of the presence of Terpenoids.

Test for Fats and Fixed oils

Stain Test: A small quantity of sample was pressed between two filter papers. A stain on the filter paper is an indication of the presence of fixed oils.

3.3 *IN-VITRO* STUDY FOR DIURETIC ACTIVITY

3.3.1 Carbonic Anhydrase Inhibition Assay (S.I. Ibrahim, *et al* 2016) (9)

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 assay was conducted in HEPES (Hydroxy ethyl piperazine ethane sulfonic acid)-Tris buffer of pH 7.4 (20 mM) at 25°C. Each inhibitory tube is consisted of 1400 µL of HEPES-Tris buffer solution, 400 µL of Carbonic Anhydrase enzyme solution (0.1 mg/mL HEPES-Tris buffer), and 400 µL of test compound in HPLC grade Dimethyl Sulfoxide (maintain10% of the final concentration). The mixture solution was pre-incubated for 15 min at 25°C. Substrate *p*-nitro phenyl acetate (*p*-NPA) (0.7 mM) was prepared in HPLC grade methanol and the reaction was started by adding 400 µL to each tube. The amount of product formed was measured at 400 nm in a UV spectrophotometer, Elico (India). The activity of the controlled compound was taken as 100%. All experiments were carried out in triplicates of each used concentration, and results was represented as a mean of the triplicate. Acetazolamide was used as standard.

3.4 *IN VIVO* DIURETIC ACTIVITY

3.4.1 EXPERIMENTAL ANIMALS

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/20.09.2021 and 04/AEL/IAEC/MMC, Date :26/12/2023. Albino Wistar Rats was used for this study and was procured from Animal house, Madras Medical College, Chennai, India.

3.4.2 SELECTION OF ANIMAL SPECIES

The preferred rodent species was the Albino Wistar Rat, although other rodent species may be used. Healthy young adult animals were commonly used laboratory strains to be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (100-200gm) should fall in interval within ±20% of the mean weight of any previously dosed animals. Sexually matured Albino Wistar Rats were obtained from Animal Experiments Laboratory, Madras Medical College, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet.

3.4.3 HOUSING AND FEEDING CONDITIONS

The temperature in the animal experimental room should be 22±3°C. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

3.4.4 ANIMAL IDENTIFICATION

All animal cages were 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.

3.4.5 PREPARATION OF ANIMALS

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions. Rats were deprived of food overnight (but not water 16-18h) prior to administration of the *Pongamia pinnata*.

Test substance	: *Pongamia pinnata* Ethanolic Seed Extract (PPESE)
Animal source	: Animal Experimental Laboratory,MMC,
Animals	: Albino Wistar Rats
Age	: 6-8 weeks
Gender	: Either Sex
Body weight	: 100-200g
Acclimatization	: Seven days prior to dosing
Veterinary examination	: Prior and at the end of the acclimatization period
Identification of animals	: By cage number and individual marking by picric acid.
Number of animals	: 24 Rats
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.

3.4.6 ACUTE TOXICITY STUDY (10) (S B Dahikar, et al.)

Acute toxicity study had been carried out already and the animals did not show any toxic effects up to dose of 2000 mg/kg as per OECD guidelines – 423. Hence 1/10 th and 1/5th of the maximum dose administered (i.e., 200 mg/kg and 400 mg/kg) was selected for this study.

3.4.7 GROUPING OF ANIMALS

Totally 24 Albino Wistar Rats (mix of male and female) were used in this study. These animals were divided into 4 groups, each containing 6 animals.

3.4.8 EVALUATION OF DIURETIC ACTIVITY (Amuthan.A *et al.* 2012) (11)

Method:

Diuretic activity in Albino Wistar Rats by LIPSCHITZ method.

Procedure:

Animals were fasted overnight before the start of study but provided with drinking water *ad libitum*. Animals in group I, II, III and IV were treated with vehicle, standard and two different doses of test drugs respectively. Immediately after dosing, animals were caged into separate metabolic cages for urine collection. After 24 hours, urine samples were collected and tested for diuretic activity.

3.4.9 EXPERIMENTAL DESIGN

Table :2

GROUP NO.	GROUP	TREATMENT AND ROUTE OF ADMINISTRATION	NO. OF ANIMALS
Group I	Control	Distilled water(5ml/kg) p.o.	6
Group II	Standard	Hydrochlorothiazide (10mg/kg) p.o.	6
Group III	Treatment Control (Low Dose)	*Pongamia pinnata* Ethanolic Seed extract (200 mg/kg) p.o.	6
Group IV	Treatment Control (High Dose)	*Pongamia pinnata* Ethanolic Seed extract (400 mg/kg) p.o.	6
TOTAL NO. OF ANIMALS REQUIRED			24 Animals

3.5 EVALUATION PARAMETERS

3.5.1 Urine volume

Drug treated animals were placed metabolic cage and within 24 hours, urine samples were collected from each group. Then the collected urine samples were measured separately, by using calibrated measuring cylinder.

3.5.2 Urinary pH

All the animal's urine samples were measured separately, by using glass electrode pH meter for the estimation of urinary pH.

3.5.3 Estimation of urinary electrolyte

Animal's urine samples were analyzed for sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions concentrations respectively by Ion Selective Electrode method.

3.6 STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (SEM). All data was analysed by one-way analysis of variance test (ANOVA), followed by Dunnett's multiple comparison test using Graph Pad Prism, version (8.0.2), with level of significance set as $P<0.05$, $P< 0.01$ and $P < 0.001$ versus control.

4. RESULTS AND DISCUSSION

4.1 EXTRACTION

The Percentage Yield of the Ethanolic Extract of the *Pongamia pinnata* Seed obtained through Soxhlet Extraction was found to be **18 % w/w**.

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4.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table 3: Phytochemical analysis of PPESE

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.	Glycosides	Legal's Test	+
		Keller Killiani Test	+
4.	Saponin	Froth Test	+
		Foam Test	+
5.	Steroids	Liebermann Burchard Test	+
6.	Phenols	Ferric chloride Test	+
7.	Tannins	Gelatin Test	+
		Lead Acetate Test	+
8.	Flavonoids	Alkaline reagent test	+
9.	Proteins and amino acid	Millon's Test	+
10.	Fats and Fixed oils	Stain Test	+

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

4.3 *IN VITRO* ACTIVITY

4.3.1 Carbonic Anhydrase Inhibition Assay

The carbonic anhydrase inhibition activity of the test drug **PPESE** and standard drug, **Acetazolamide** was found to be **113.42 µg/ml** and **51.29 µg/ml** respectively.

Table 4: Percentage (%) Inhibition of standard drug- Acetazolamide

CONCENTRATION (µg)	ABSORBANCE			%INHIBITION
	SINGLET	DOUBLET	TRIPLET	
0	0.92	0.93	0.92	0
10	0.56	0.54	0.56	40.1
20	0.43	0.42	0.43	53.9
40	0.35	0.34	0.36	62.1
80	0.24	0.26	0.24	73.4
160	0.14	0.15	0.16	83.8
320	0.08	0.087	0.089	90.8

Table 5: Percentage (%) Inhibition of Test drug- PPESE

CONCENTRATION (µg)	ABSORBANCE			%INHIBITION
	SINGLET	DOUBLET	TRIPLET	
0	0.98	0.97	0.98	0
10	0.717	0.717	0.717	26.08
20	0.657	0.657	0.657	32.26
40	0.632	0.632	0.632	34.84
80	0.494	0.494	0.494	49.07
160	0.206	0.206	0.206	78.76
320	0.106	0.106	0.106	89.07

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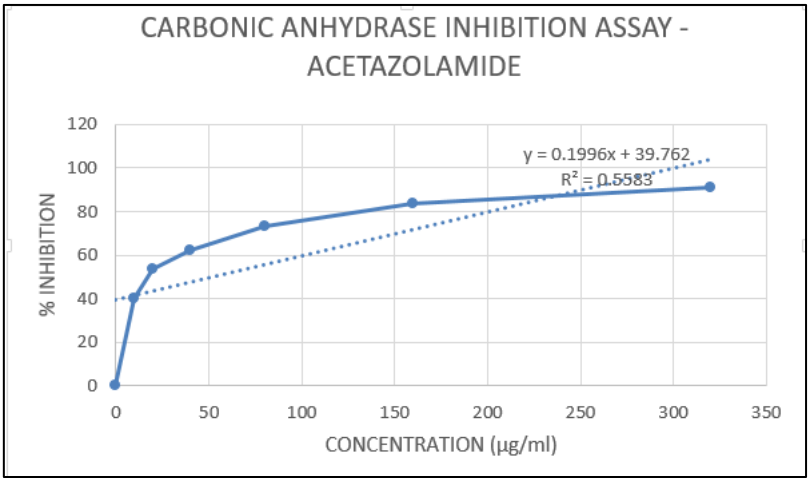

Fig 1: CAI Assay Vs Acetazolamide Graphical Representation

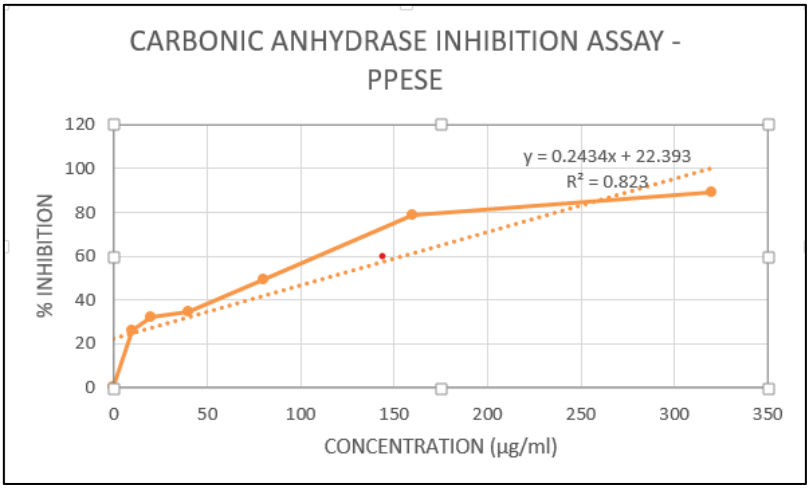

Fig 2: CAI Assay Vs PPESE Graphical Representation

4.4 *IN VIVO* ACTIVITY

4.4.1 EVALUATION PARAMETERS

4.4.1.1 Urine volume

Effect of **PPESE** on urine volume was assessed and the results were depicted in the **table 6** and **figure 3**.

Table 6: Effect of PPESE on urine volume

S.NO	GROUP	URINE VOLUME (ml)
1	Normal (Distilled water)	4.75±0.05
2	Standard (Hydrochlorothiazide)	7.36±0.12****
3	Low dose of PPESE (200mg/kg)	4.4±0.10*
4	High dose of PPESE (400mg/kg)	5.35±0.07***

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

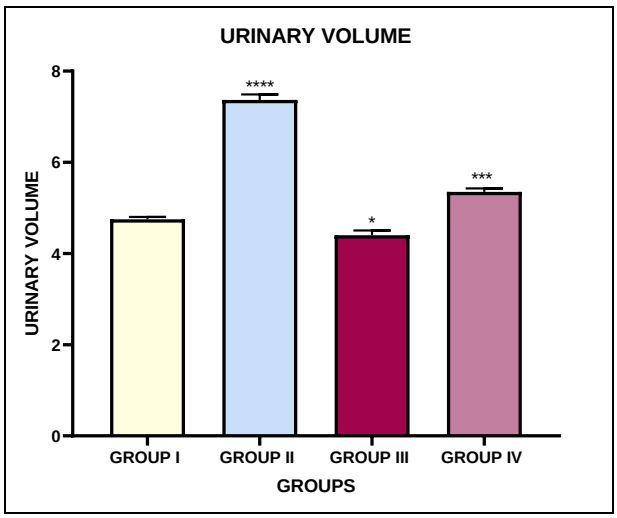

Figure 3: Effect of PPESE on urinary volume

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 **7.3 ± 0.12**. 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 **4.4 ± 0.10**. It shows that there was slight increase (P < 0.05) in urine volume when compared to normal control group. At the higher dose (400mg/kg), urine volume was found to be **5.35 ± 0.07** and showed significant increase (P < 0.001) 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.

4.4.1.2 Urinary pH

Effect of PPESE on urinary pH was determined and the results were given in the **table 7** and **figure 4**.

Table 7: Effect of PPESE on Urinary pH

S.NO	GROUP	URINARY pH
1	Normal (Distilled water)	6.68±0.0
2	Standard (Hydrochlorothiazide)	7.58±0.07****
3	Low dose of PPESE (200mg/kg)	5.94±0.02****
4	High dose of PPESE (400mg/kg)	6.41±0.06**

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All values are expressed as Mean ± SEM, (n=6), **** P< 0.0001, ** P< 0.01, when compared to normal control group.

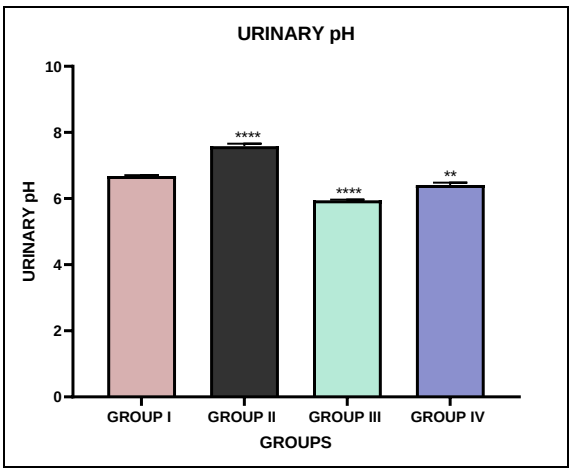

Figure 4: Effect of PPESE on urinary pH

All values are expressed as Mean ± SEM, (n=6), **** P<0.0001, ** P< 0.01, when compared to normal control group.

The results stated above confirmed 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 7.58 ± 0.02. 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 5.94 ± 0.07. It showed that there was significant increase (P < 0.0001) in urine pH when compared to normal control group. At the higher dose (400mg/kg), urinary pH was found to be 6.41± 0.06 and showed significant increase (P < 0.01) in urinary pH when compared to normal control group. Among the low dose and high dose treated groups, the higher dose treated group showed increased urinary pH which was nearly equivalent to standard drug treated group.

4.4.1.3 Estimation of Urinary electrolyte

Effect of **PPESE** on urinary sodium (Na^+) was assessed and the results were depicted in the **table 8** and **figure 5, 6 and 7**.

Table 8: Effect of PPESE on Urinary electrolyte

S. NO	GROUP	SODIUM (Na+) (mEq/L)	POTASSIUM (K+) (mEq/L)	CHLORIDE (Cl-) (mEq/L)
1	Control (Distilled water)	80.3±0.13	65.29±0.04	90.37±0.10
2	Standard (Hydrochlorothiazide)	91.51±0.44****	75.35±0.06****	100.58±0.13****
3	Low dose of PPESE (200mg/kg)	81.54±0.15*	68.18±0.06****	91.34±0.06****
4	High dose of PPESE (400mg/kg)	83.35±0.08****	70.68±0.32****	94.41±0.06****

All values are expressed as Mean ± SEM, (n=6), **** P < 0.0001, *** P < 0.001 and ** P < 0.01, when compared to normal control group.

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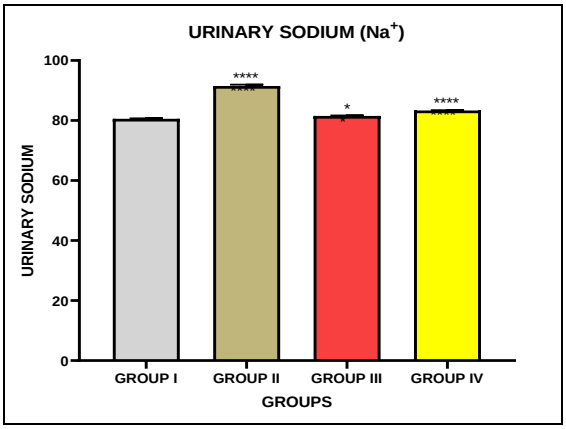

Figure 5: Effect of PPESE on sodium excretion

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

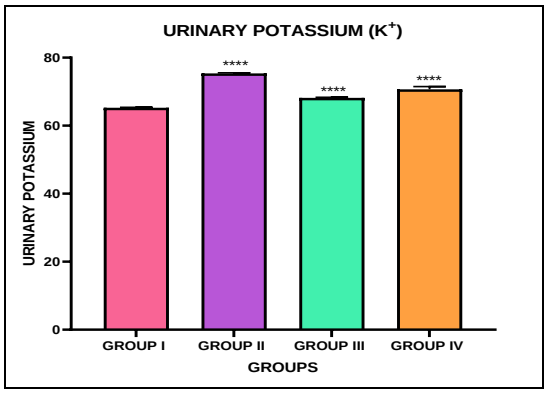

Figure 6: Effect of PPESE on potassium excretion

All values are expressed as Mean ± SEM, (n=6), ** P < 0.01, when compared to normal control group.

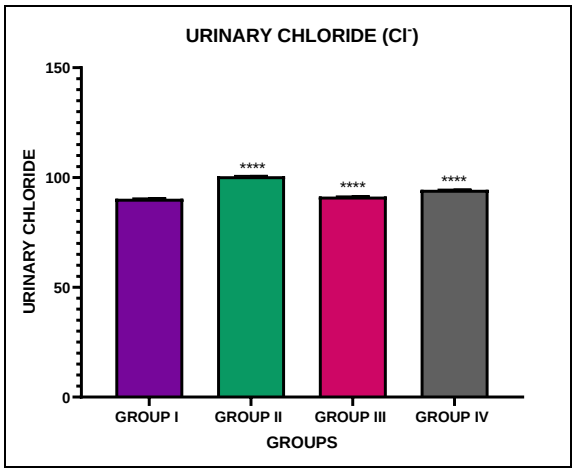

Figure 7: Effect of PPESE on chlorides excretion

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

The results confirmed 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 91.51 ± 0.44 mEq/L

with significance of (P < 0.0001) for sodium, 73.35± 0.06 mEq/L with significance of (P < 0.0001) for potassium and 100.58 ± 0.13 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 81.54 ± 0.15 mEq/L with significance of (P < 0.05) for sodium, 68.18± 0.06 mEq/L with significance of (P < 0.0001) for potassium and 91.34 ± 0.06 mEq/L with significance of (P < 0.0001) for chloride ion respectively, when compared to normal control group. At the higher dose (400mg/kg), electrolyte excretion was found to be 83.35±0.08mEq/L with significance of (P < 0.0001) for sodium, 70.68±0.32 mEq/L with significance of (P < 0.0001) for potassium and 94.41±0.06 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) showed the increased electrolyte excretion which was nearly equivalent to standard drug treated group.

CONCLUSION

Pongamia pinnata is a plant medicine known for its diuretic properties. It is commonly used to treat conditions such as oedema, high blood pressure, and certain kidney disorders. While its diuretic effects have been acknowledged in traditional medicine, there is a lack of extensive scientific research and clinical trials on PPESE. This study aims to bridge that gap by conducting scientific investigations.

Phytochemical screening evidenced the presence of various compounds in PPESE, including alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, and saponins. These compounds contribute to its potential health benefits and therapeutic properties. Alkaloids have known pharmacological activities, such as analgesic and anti-inflammatory effects. Glycosides often exhibit cardiovascular and anti-cancer properties. Carbohydrates provide energy and support metabolic processes. Phenolic compounds, tannins, and flavonoids act as antioxidants, protecting cells from oxidative stress and reducing the risk of chronic diseases. Saponins have potential as natural surfactants. The presence of fats and fixed oils indicates lipid content in PPESE. These primary phytochemicals contribute to the various kind of biological activities of PPESE.

In vitro study was conducted to assess the diuretic property of PPESE. It displayed Carbonic anhydrase inhibitory potential of the test drug. While acetazolamide showed higher inhibitory activity at a lower concentration, the study confirmed the in vitro diuretic activity of PPESE. Further evaluation, including bioavailability, pharmacokinetics, and safety profiles, is required to determine its clinical potential and efficacy for treating conditions where carbonic anhydrase inhibition is desirable.

In vivo study was conducted to further evaluation of the *Pongamia pinnata* in Animals. In acute oral toxicity study, there was no sign of adverse effects or toxicity observed in experimental animals. No mortality or morbidity was observed during the study period. This study provided initial safety information, to select the low and high doses respectively, for further in vivo studies. Diuretic activity was assessed in animals by measuring urine volume, urinary pH, electrolyte excretion.

Both the low and high doses of PPESE exhibited significant increase in urine volume compared to the control group. The higher dose of PPESE also indicated similar effects on urinary pH and electrolyte excretion as the standard drug.

The study confirms, that PPESE, when taken orally in a single dose, exhibits diuretic potential. It promotes salt excretion and increases urine output in a dose-dependent manner, resulting in the alkalinization of urine. These findings suggest that the PPESE may act as a thiazide diuretic, as it significantly increases the excretion of sodium, potassium, and chloride ions. The diuretic activity is attributed to the presence of phytochemicals, including saponins, flavonoids, phenolic compounds, and steroids in the PPESE. Saponins are believed to facilitate sodium elimination by modulating renal sodium content, while phenolic compounds, organic acids, flavonoids and steroids contribute to the diuretic effects of PPESE.

In conclusion, the study provides scientific evidence supporting the diuretic properties of PPESE. The physico-chemical analysis revealed important characteristics such as pH. Phytochemical screening identified the presence of various compounds known for their therapeutic benefits, including alkaloids, glycosides, saponins, phenolic compounds, tannins, and flavonoids. In vitro studies confirmed Carbonic Anhydrase Inhibition potential.

In vivo study demonstrated the safety of PPESE and its diuretic effects in animals, indicating increased urine volume, alkalinization of urine with electrolyte excretion. These findings suggest that PPESE may act as a thiazide diuretic, primarily due to the presence of saponins, flavonoids, phenolic compounds, and steroids., etc. However, further research is required to fully understand its exact mechanism of action, bioavailability, pharmacokinetics, and clinical efficacy for treating conditions where diuretic effects are essential. Overall, this study contributes to the scientific knowledge on PPESE and supports its traditional claim as a Diuretic agent.

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Conflict of interest statement: The authors declared no conflict of interest.

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