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NEPHROPROTECTIVE ACTIVITY OF *TAMARINDUS* AND *PITHECELLOBIUM DULCE* FRUITS EXTRACT IN EXPERIMENTAL RATS

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ABSTRACT
This study evaluated the nephroprotective potential of *Tamarindus indica* and *Pithecellobium dulce*, individually and in combination, through phytochemical analysis, acute toxicity assessment, and in vivo renal function studies. Plant materials were collected, authenticated, and subjected to methanolic, aqueous, and petroleum ether extraction. Phytochemical screening confirmed the presence of bioactive compounds, including phenols, flavonoids, alkaloids, tannins, proteins, and glycosides. Acute oral toxicity studies demonstrated that the extracts were safe up to 2000 mg/kg, with no observable adverse effects. Polyherbal and single-plant formulations were administered to Wistar rats, resulting in improved renal parameters, including enhanced urine output and reduced serum creatinine and blood urea nitrogen levels. Histopathological examination supported these findings, revealing preservation of glomerular and tubular structures, particularly in *Pithecellobium dulce*-treated groups. Overall, the results validate the traditional use of these plants for renal protection, highlight the superior efficacy of *Pithecellobium dulce*, and provide a scientific basis for the development of safe, effective herbal formulations targeting kidney health.

KEYWORDS: *Tamarindus indica*, *Pithecellobium dulce*, Nephroprotection, Phytochemical screening, Serum creatinine, Blood urea nitrogen.

1. INTRODUCTION
Herbal medicine has long served as a vital resource in the prevention and management of various diseases due to its rich bioactive compounds and relatively low toxicity (Verma, Singh, & Garg, 2016). Medicinal plants are a source of secondary metabolites such as phenols, flavonoids, tannins, saponins, alkaloids, and glycosides, which contribute to multiple pharmacological activities, including nephroprotection, anti-inflammatory effects, and free radical scavenging (Fouad, Jresat, & Hegazy, 2018). The increasing incidence of renal disorders and the limitations of conventional therapies have driven interest in exploring plant-based remedies for kidney health (Arfat, Singh, & Sharma, 2014).

Tamarindus indica L. (Fabaceae), commonly known as tamarind, is widely distributed in tropical regions and has been traditionally used for its hepatoprotective, antidiabetic, anti-inflammatory, and laxative properties (Akinmoladun, Oyeleye, & Adeyemi, 2019). Phytochemical studies of *T. indica* reveal a diverse range of bioactive compounds, including flavonoids, phenolic acids, tannins, saponins, and glycosides, which contribute to its therapeutic potential (Ogbonna, Okoye, & Nwafor, 2020). These compounds are known to protect renal tissues by modulating oxidative stress, inflammation, and tissue damage.

Pithecellobium dulce (Fabaceae), also referred to as Manila tamarind or Madras thorn, has been used in traditional medicine for its anti-inflammatory, antidiabetic, and antimicrobial properties (Kumar, Singh, & Sharma, 2021). Its leaves, bark, and seeds are rich in phenolic compounds, flavonoids, and alkaloids, which exhibit nephroprotective effects by supporting renal

function and reducing biomarkers of kidney injury (Patil, Jadhav, & Shinde, 2020).

Given the complementary phytochemical profiles and traditional uses of *T. indica* and *P. dulce*, their combination in polyherbal formulations may offer synergistic nephroprotective benefits. This study investigates the phytochemical composition, safety, and renal protective effects of these plants individually and in combination, providing scientific validation for their use in kidney health management.

2. Collection and Authentication of Plant Material

Plant materials of *Tamarindus indica* and *Pithecellobium dulce* were collected from regions of traditional medicinal use, authenticated by a qualified botanist, and voucher specimens deposited in an herbarium. The materials were washed, shade-dried to preserve phytochemicals, coarsely powdered, and stored in airtight containers for subsequent extraction and analysis.

2.1 Extraction of Plant Material by Soxhlation Process

The coarsely powdered plant material of *Tamarindus indica* and *Pithecellobium dulce* was extracted using a Soxhlet apparatus with solvents of varying polarity (aqueous, petroleum ether, and methanol). Extraction was continued for 6–8 hours until the solvent became clear, followed by filtration and concentration using a rotary evaporator. The crude extracts were dried, stored in airtight containers, and the percentage yield was calculated to ensure efficiency and purity for further phytochemical and pharmacological evaluation. The percentage yield of *Tamarindus indica* and *Pithecellobium dulce* extracts was calculated using the following:

(%) Yield = (Weight of extract/ Weight of Plant Material used) □100

2.2 Phytochemical Test of *Tamarindus and pithecellobium dulce*by qualitative analysis

➢ **Test for Carbohydrates**

1. **Molisch's Test:** A 1 mL aqueous extract of *Tamarindus indica* and *Pithecellobium dulce* was treated with Molisch's reagent (α-naphthol) followed by careful addition of concentrated sulfuric acid along the test tube wall. The appearance of a violet or purple ring at the interface confirmed the presence of carbohydrates in the extracts.
2. **Fehling's Test:** Equal volumes (1 mL each) of Fehling's solutions A and B were mixed, and 2 mL of the aqueous extract of *Tamarindus indica* and *Pithecellobium dulce* was added. Upon heating for 5–10 minutes, the formation of a reddish-brown precipitate of cuprous oxide indicated the presence of reducing sugars in the extracts.
3. **Benedict's test:** Equal volumes of Benedict's reagent and the aqueous extracts of *Tamarindus indica* and *Pithecellobium dulce* were mixed and heated for 5–10 minutes. The appearance of green, yellow, or red coloration, depending on sugar concentration, confirmed the presence of reducing sugars in the extracts.
4. **Barfoed's Test:** One milliliter of the aqueous extract of *Tamarindus indica* and *Pithecellobium dulce* was mixed with Benedict's reagent and boiled. The development of a red color, due to cupric oxide formation, confirmed the presence of monosaccharides in the extracts.
5. **Froth Test:** One milliliter of *Tamarindus indica* and *Pithecellobium dulce* extracts was mixed with distilled water and shaken vigorously. The formation of stable foam indicated the presence of saponins in the extracts.

➢ **Test for Tannin and Phenolic Compounds**

1. **Ferric Chloride Test:** The extracts of *Tamarindus indica* and *Pithecellobium dulce* were dissolved in distilled water and treated with a few drops of diluted ferric chloride solution. The appearance of a deep blue color confirmed the presence of tannins.
2. **Gelatin Test:** A measured amount of *Tamarindus indica* and *Pithecellobium dulce* extracts was mixed with distilled water, followed by the addition of 2 mL of 1% gelatin solution containing 10% sodium chloride. The formation of white precipitates indicated the presence of phenolic compounds.
3. **Lead Acetate Test:** A small quantity of *Tamarindus indica* and *Pithecellobium dulce* extracts was mixed with distilled water and treated with a few drops of lead acetate solution. The formation of a white precipitate confirmed the presence of phenolic compounds.

➢ **Test for Triterpenoids and Steroids**

1. **Libermann-Burchard Test:** The extracts of *Tamarindus indica* and *Pithecellobium dulce* were dissolved in chloroform, mixed with 1 mL each of acetic acid and acetic anhydride, and heated in a water bath. After cooling, a few drops of concentrated sulfuric acid were added along the test tube walls. The appearance of a blue-green color confirmed the presence of steroids.
2. **Salkowski Test:** The extracts of *Tamarindus indica* and *Pithecellobium dulce* were dissolved in chloroform and mixed with an equal volume of concentrated sulfuric acid. The presence of steroids was indicated by a bluish-red to cherry-red color in the chloroform layer and green fluorescence in the acid layer.

➢ **Test for Glycosides**

1. **Borntragers Test:** Three milliliters of the test solution were mixed with diluted sulfuric acid and boiled for five minutes. After cooling, an equal volume of benzene or chloroform was added, shaken, and the organic layer separated. Treatment with ammonia produced a pink to crimson color, indicating the presence of anthraquinone glycosides.
2. **Keller Killiani Test:** Two milliliters of the test solution were mixed with 3 mL of glacial acetic acid

and a drop of 5% ferric chloride solution, followed by the careful addition of 0.5 mL concentrated sulfuric acid. The development of a blue color in the acetic acid layer confirmed the presence of cardiac glycosides.

➢ **Test for protein and amino acids**

1. **Biuret's test:** The extract was mixed with 1 mL of 10% sodium hydroxide solution and heated, followed by the addition of a drop of 0.7% copper sulfate solution. The development of a violet or pink color indicated the presence of proteins.
2. **Ninhydrin test:** Three milliliters of the extract were heated with three drops of 5% ninhydrin solution in a water bath for ten minutes. The appearance of a blue color confirmed the presence of amino acids.

➢ **Tests for Alkaloids**

1. **Dragendorff's Test:** One milliliter of *Tamarindus indica* and *Pithecellobium dulce* extract was mixed with alcohol, a few drops of acetic acid, and Dragendorff's reagent. The formation of an orange-red precipitate confirmed the presence of alkaloids.
2. **Wagner's Test:** One milliliter of *Tamarindus indica* and *Pithecellobium dulce* extract was dissolved in acetic acid, and a few drops of Wagner's reagent were added. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.
3. **Mayer's Test:** One milliliter of *Tamarindus indica* and *Pithecellobium dulce* extract was mixed with acetic acid and treated with a few drops of Mayer's reagent. The appearance of a dull white precipitate suggested the presence of alkaloids.
4. **Hager's Test:** About 1–2 mL of *Tamarindus indica* and *Pithecellobium dulce* extract was mixed with acetic acid and 3 mL of Hager's reagent. The formation of a yellow precipitate confirmed the presence of alkaloids.

➢ **Test for Flavonoids**

1. **Lead Acetate test:** The extract was treated with a few drops of lead acetate solution. The appearance of a yellow precipitate suggested the possible presence of flavonoids.
2. **Alkaline reagent test:** The extract was treated with a few drops of sodium hydroxide solution in a test tube. The appearance of a bright yellow color, which turned colorless upon adding diluted acid, indicated the presence of flavonoids (Yadav & Agarwala, 2011).

2.3 Determination of total phenols content (TPC) by spectrophotometric method

The total phenolic content (TPC) of *Tamarindus indica* and *Pithecellobium dulce* extracts was measured using the Folin–Ciocalteu method. A 40 µL aliquot of extract (1 mg/mL) or gallic acid standard was mixed with 200 µL Folin–Ciocalteu reagent, diluted to 3.16 mL with distilled water, and allowed to react for 8 minutes. Subsequently, 600 µL of sodium carbonate was added, and the mixture was incubated at 40 °C for 30 minutes. Absorbance was recorded at 760 nm, and phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of extract using a gallic acid calibration curve (20–100 µg/mL) (Singleton & Rossi, 2009).

2.4 Determination of total flavonoid content (TFC) by spectrophotometric method

The total flavonoid content of *Tamarindus indica* and *Pithecellobium dulce* extracts was measured using the aluminum chloride colorimetric method. A 0.2 g sample was dissolved in 1 mL distilled water, and 0.5 mL of this solution was mixed with 1.5 mL 95% ethanol, 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M potassium acetate, and 2.8 mL distilled water. After incubating at room temperature for 40 minutes to form a flavonoid–aluminum complex, absorbance was recorded at 510 nm. A rutin calibration curve (20–100 µg/mL) was used to calculate total flavonoid content, expressed as mg rutin equivalents (RE) per gram of extract (Lin & Tang, 2007).

2.5 Preparation of polyherbal suspension

The three herbal suspensions were formulated using a standardized base of sodium carboxymethyl cellulose (2 g) as a suspending agent, Tween 80 (0.1%) for improved dispersion, and methyl paraben (0.2%) as a preservative. Sweeteners, sucrose (10 g) and sorbitol (5 g), were added to enhance palatability. Formulation I contained 1 g *Tamarindus indica*, Formulation II 1 g *Pithecellobium dulce*, and Formulation III combined both extracts (1 g each) in a polyherbal mixture. All formulations were adjusted to 100 mL with distilled water and thoroughly mixed to ensure uniformity. This standardized composition allows consistent comparison in pharmacological evaluations (Jyoti et al., 2012).

Table 3: Composition of prepared suspension.

Name of Ingredient	Formulation I	Formulation II	Formulation III
***Tamarindus indica*(Ratio)(250mg)**	1.0	---	1.0
***Pithecellobium dulce*(Ratio) (250mg)**	---	1.0	1.0
Tween 80	0.1%	0.1%	0.1%
Sodium CMC	2.0 g	2.0 g	2.0 g
Sucrose	10 g	10 g	10 g
Sorbitol	5.0 g	5.0 g	5.0s g
Methyl parabeen	0.2%	0.2%	0.2%
Distilled water q. s.	100 ml	100 ml	100 ml

2.6 Physicochemical Parameters of Formulated Suspensions

Quality control of the prepared polyherbal suspensions included evaluation of physicochemical parameters such as pH, viscosity, sedimentation volume, and redispersibility to ensure formulation consistency, stability, and overall acceptability.

2.6.1 pH Measurement

The pH of each suspension was measured using a digital pH meter. One gram of each formulation was dispersed in 100 mL distilled water and equilibrated for 2 hours at room temperature. Measurements were performed in triplicate, and the average values were recorded to assess reproducibility and formulation stability.

2.6.2 Viscosity

The viscosity of the suspensions was measured at 100 rpm using a Brookfield viscometer with the appropriate spindle. Measurements were conducted at room temperature to evaluate the rheological properties and pourability of the formulations.

2.6.3 Skin Irritation Studies (For Suspensions Intended for Dermal Use)

For dermal application, a skin irritation study was conducted using Wistar rats (180–200 g, either sex). The dorsal fur was shaved 72 hours prior, and each suspension was applied topically once daily for 7 days. A base formulation without extract served as the control. The treated sites were examined for redness, edema, or other reactions to evaluate potential skin irritancy.

2.7 Acute Toxicity Study

Acute toxicity was evaluated following OECD Guideline 423 using a stepwise approach with three animals per phase, with ethical approval from the Faculty Ethical Committee. Oral administration of methanolic extracts of *Tamarindus indica* and *Pithecellobium dulce* at doses of 5, 50, 300, and 2000 mg/kg over 14 days caused no mortality, adverse effects, or abnormal clinical signs. Body weight and food intake remained comparable to controls, indicating safety at the tested doses (Vysakh et al., 2020).

2.8 *In vivo* study of Nephroprotective activity in rats

2.8.1 Experimental work

The study was approved by the Institutional Animal Ethics Committee (IAEC, Approval No. PBRI/IAEC/07-04-25/013) and conducted following its guidelines. Wistar albino rats (200 ± 20 g) were obtained from the Pinnacle Biomedical Research Institute (PBRI) and housed under conditions complying with institutional standards.

2.8.1.1 Experimental modelling

During acclimatization, rats were fed Golden Feed (Bhopal) and given water ad libitum. They were maintained at 22 ± 1 °C, 40–60% humidity, and a 14-hour light/10-hour dark cycle for one week. Water was withheld for 16 hours and food restricted before experiments, and all surgical procedures were performed under sterile conditions.

2.8.1.1.1 Drugs used for nephrotoxicity

- **Paracetamol**

Animals received a paracetamol suspension orally at 750 mg/kg body weight via gastric tube. The suspension was prepared by dissolving paracetamol in 1% carboxymethyl cellulose in phosphate-buffered saline, with each milliliter containing 750 mg of the drug.

- **Silymarin**

A 40 mg dose of Silymarin was dissolved in 0.9% saline solution adjusted to pH 7.0. The animals received this Silymarin solution orally via gastric tube at a dosage of 40 mg/kg.

2.8.1.1.2 Experimental protocol

Five groups were randomly selected from among the animals, with six rats in each group.

Group	Description	Treatment
Group I	Control group	No treatment (untreated control)
Group II	Paracetamol group (Inducer)	Oral administration of paracetamol (750 mg/kg body weight)
Group III	SilymarinStandard treatment group	Daily oral administration of Silymarin(40 mg/kg body weight)
Group IV	Formulation I	Rats were treated with *Tamarindus indica* suspension (Formulation I) containing 250mg of extract.
Group V	Formulation II	Rats were treated with *Pithecellobium dulce* suspension (Formulation II) containing 250mg of extract
Group VI	Formulation III	Rats were treated with polyherbal suspension (Formulation III)

All treatments were administered for 30 days, beginning four days prior to silymarin administration. Body weight of each animal was recorded weekly throughout the study.

2.8.2 Blood samples for biochemical estimation

Blood samples were collected from the retro-orbital venous plexus into non-heparinized tubes, centrifuged at 3000 rpm for 20 minutes, and the serum was separated. The serum was stored at 4 °C until biochemical analysis using Erba's ready-to-use kits.

2.9 Analysis of general parameters

2.9.1 Analysis of urine

After the final treatment, animals were placed individually in metabolic cages for 24 hours to collect urine and measure volume. Urine samples were analyzed for glucose and protein using standard diagnostic kits (Azab et al., 2016).

2.9.2 Estimation of Body weight

At the end of the study, animals were housed separately, with food and water withheld, and each was individually weighed. The recorded weights were documented for further analysis.

2.9.3 Serum Creatinine and blood urea nitrogen (BUN) analysis

Plasma blood urea nitrogen (BUN) and creatinine levels were measured to assess renal function. Samples were spiked with 10 µL of creatinine standard or 0.2 N HCl as controls. While both BUN and serum creatinine are used to evaluate renal function, BUN is less sensitive for detecting mild to moderate impairment. BUN was determined using a biochemical analyzer: urease hydrolyzed urea to ammonia and carbon dioxide, followed by glutamate dehydrogenase–catalyzed conversion of ammonia and α-ketoglutarate to glutamate and NAD, with the decrease in absorbance at 340 nm (due to NADH oxidation) used to quantify BUN levels (Arfat et al., 2014).

3. RESULTS

3.1 Procurement of plant material

Percentage yield analysis of *Tamarindus indica* and *Pithecellobium dulce* across methanol, petroleum ether, and aqueous solvents showed distinct extraction efficiencies. For *Tamarindus indica*, yields were 7.92% (methanol), 7.00% (petroleum ether), and 7.64% (aqueous), with methanol slightly outperforming other solvents. *Pithecellobium dulce* exhibited higher yields: 9.81% (methanol), 7.87% (aqueous), and 6.96% (petroleum ether), indicating methanol as the most efficient solvent for both plants. Overall, *Pithecellobium dulce* consistently yielded more extract, suggesting a richer phytochemical profile, while the aqueous extract also performed well, reflecting significant water-soluble constituents. These findings are visually represented in Figure 9.

Table 4: Percentage Yield determination of plant material.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Tamarindus indica	Methanol	450	35.67	7.92
Tamarindus indica	Petroleum ether	365	25.57	7.00
Tamarindus indica	Aqueous	371	28.35	7.64

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Pithecellobium dulce	Methanol	300	29.45	9.81
Pithecellobium dulce	Petroleum ether	284	19.78	6.96
Pithecellobium dulce	Aqueous	292	22.99	7.87

Figure 10: Yield of plant material of *Tamarindus and pithecellobium dulce*.

3.2 Phytochemical Test

Table 5: Phytochemical test of *pithecellobium dulce*and *Tamarindus* Aqueous extract.

S. No.	Experiment	Aqueous extract of pithecellobium dulce	Aqueous extract *Tamarindus*
Test for Carbohydrates			
1.	Molisch's Test	Absent	Present
2.	Fehling's Test	Absent	Present
3.	Benedict's Test	Absent	Present
4.	Bareford's Test	Absent	Present

Test for Alkaloids			
1.	Mayer's Test	Present	Present
2.	Hager's Test	Present	Present
3.	Wagner's Test	Present	Present
4.	Dragendroff's Test	Present	Present
Test for Terpenoids			
1.	Salkowski Test	Absent	Absent
2.	Libermann-Burchard's Test	Absent	Absent
Test for Flavonoids			
1.	Lead Acetate Test	Present	Present
2.	Alkaline Reagent Test	Present	Present
3.	Shinoda Test	Present	Present
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	Present	Present
2.	Lead Acetate Test	Present	Present
3.	Gelatine Test	Present	Present
4.	Dilute Iodine Solution Test	Present	Present
Test for Saponins			
1.	Froth Test	Present	Present
Test for Protein and Amino acids			
1.	Ninhydrin Test	Present	Present
2.	Biuret's Test	Present	Present
3.	Million's Test	Present	Present
Test for Glycosides			
1.	Legal's Test	Present	Absent
2.	Keller Killani Test	Present	Absent
3.	Borntrager's Test	Present	Absent

Table 6: Phytochemical test of *pithecellobium dulce*by petroleum ether andmethanolic extract.

S.No.	Experiment	Pet. Ether extract	Methanol extract
Test for Carbohydrates			
1.	Molisch's Test	Absent	Present
2.	Fehling's Test	Absent	Present
3.	Benedict's Test	Absent	Present
4.	Bareford's Test	Absent	Present
Test for Alkaloids			
1.	Mayer's Test	Present	Present
2.	Hager's Test	Present	Present
3.	Wagner's Test	Present	Present
4.	Dragendroff's Test	Present	Present
Test for Terpenoids			
1.	Salkowski Test	Absent	Absent
2.	Libermann-Burchard's Test	Absent	Absent
Test for Flavonoids			
1.	Lead Acetate Test	Present	Present
2.	Alkaline Reagent Test	Present	Present
3.	Shinoda Test	Present	Present
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	Present	Present
2.	Lead Acetate Test	Present	Present
3.	Gelatine Test	Present	Present
4.	Dilute Iodine Solution Test	Present	Present

Test for Saponins			
1.	Froth Test	Absent	Absent
Test for Protein and Amino acids			
1.	Ninhydrin Test	Absent	Present
2.	Biuret's Test	Absent	Present

3.	Million's Test	Absent	Present
Test for Glycosides			
1.	Legal's Test	Present	Present
2.	Keller Killani Test	Present	Present
3.	Borntrager's Test	Present	Present

Figure 11: Phytochemical test of Methanolic extract.

Table 7: Phytochemical test of *Tamarindus*bypetroleum ether and Methanolic extract.

S.No.	Experiment	Pet. Ether extract	Methanol extract
Test for Carbohydrates			
1.	Molisch's Test	Absent	Present
2.	Fehling's Test	Absent	Present
3.	Benedict's Test	Absent	Present
4.	Bareford's Test	Absent	Present
Test for Alkaloids			
1.	Mayer's Test	Absent	Present
2.	Hager's Test	Absent	Present
3.	Wagner's Test	Absent	Present
4.	Dragendroff's Test	Absent	Present
Test for Terpenoids			
1.	Salkowski Test	Absent	Absent
2.	Libermann-Burchard's Test	Absent	Absent
Test for Flavonoids			
1.	Lead Acetate Test	Present	Present
2.	Alkaline Reagent Test	Present	Present
3.	Shinoda Test	Present	Present
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	Present	Present
2.	Lead Acetate Test	Present	Present
3.	Gelatine Test	Present	Present
4.	Dilute Iodine Solution Test	Present	Present
Test for Saponins			
1.	**Froth Test**	**Absent**	**Absent**
Test for Protein and Amino acids			
1.	Ninhydrin Test	Absent	Present
2.	Biuret's Test	Absent	Present
3.	Million's Test	Absent	Present
Test for Glycosides			
1.	Legal's Test	Absent	Present
2.	Keller Killani Test	Absent	Present
3.	Borntrager's Test	Absent	Present

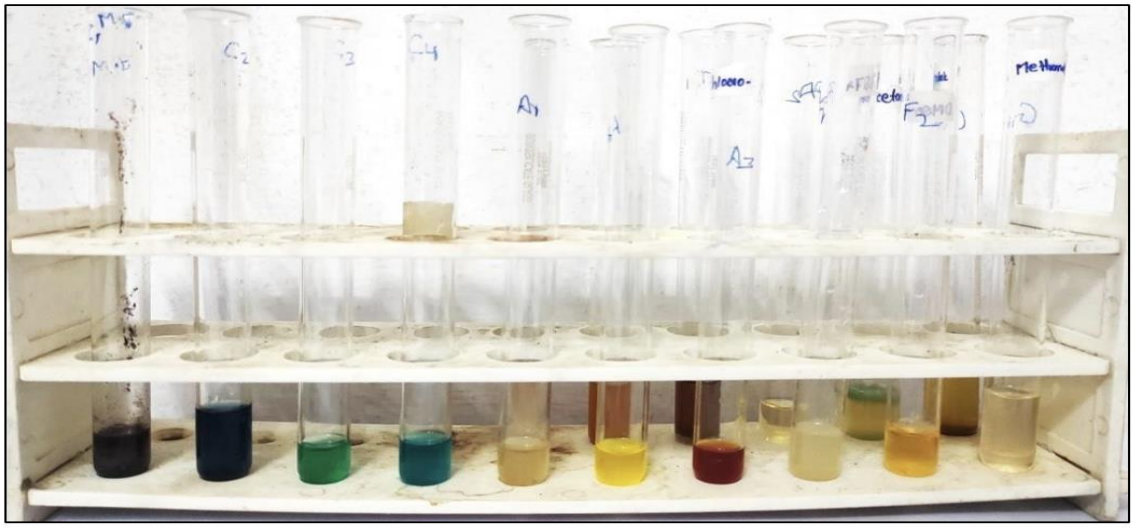


Figure 12: Phytochemical test of *Tamarindus* extract of Methanolic extract Discussion.

Phytochemical screening of *Pithecellobium dulce* and *Tamarindus indica* extracts with different solvents revealed distinct variations in bioactive compounds. Methanolic extracts were the richest, containing carbohydrates, alkaloids, flavonoids, tannins, proteins, amino acids, and glycosides, demonstrating methanol's effectiveness in extracting both polar and moderately non-polar constituents. Aqueous extracts also contained multiple compounds; however, *P. dulce* lacked carbohydrates and *T. indica* lacked glycosides, indicating limited extraction of certain constituents by water. Petroleum ether extracts, being non-polar, showed the fewest phytochemicals, mainly flavonoids and phenolics, with alkaloids detected only in *P. dulce*. Overall, methanol was the most effective solvent, followed by water, while petroleum ether exhibited limited extraction, supporting the traditional medicinal use of both plants due to their diverse therapeutic compounds.

3.3 Quatitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation

Table 8: Standard table for Gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
1.	30	0.189
2.	60	0.210
3.	80	0.238
4.	110	0.273
5.	130	0.302

Total Phenolic Content

Table 9: Total Phenolic Contentin *pithecellobium dulce*extract.

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.182	102.8mg/gm
2	0.273	
3	0.300	

Table 10: Total Phenolic Content in *Tamarindus* extract.

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.174	88.8mg/gm
2	0.250	
3	0.289	

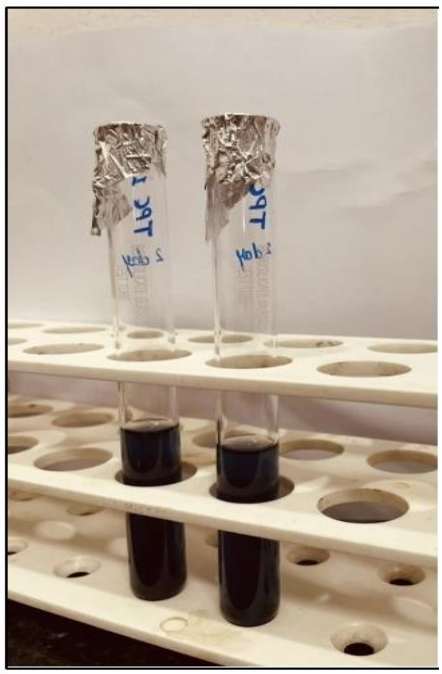


Figure 14: Total Phenolic content (TPC) estimation.

3.3.2 Total Flavonoids content (TFC) estimation

Table 11: Standard table for Rutin.

S. No.	Concentration (µg/ml)	Absorbance
1.	30	0.172
2.	60	0.198
3.	80	0.236
4.	110	0.268
5.	130	0.300

3.3.3.2 Total Flavonoid Content

Table 12: Total Flavonoid Contentin *pithecellobium dulce*extract.

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.170	87.4mg/gm
2	0.220	
3	0.260	

Table 13: Total Flavonoid Content in *Tamarindus*extract.

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.162	73.4mg/gm
2	0.204	
3	0.240	

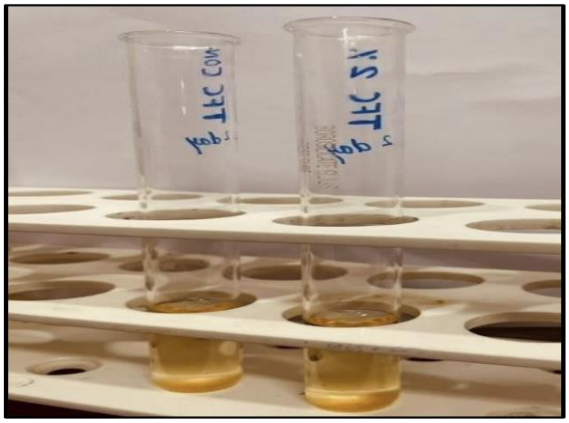


Figure 16: Total Flavonoids content (TFC) estimation.

DISCUSSION

The methanolic extract of *Pithecellobium dulce* exhibited higher total phenolic content (102.8 mg/g) and flavonoid content (87.4 mg/g) compared to *Tamarindus indica* (88.8 mg/g TPC and 73.4 mg/g TFC), indicating a potentially stronger antioxidant potential. These findings highlight the medicinal value of both plants and support further investigation into their therapeutic applications.

3.4 Paracetamol induced Nephrotoxicity Model

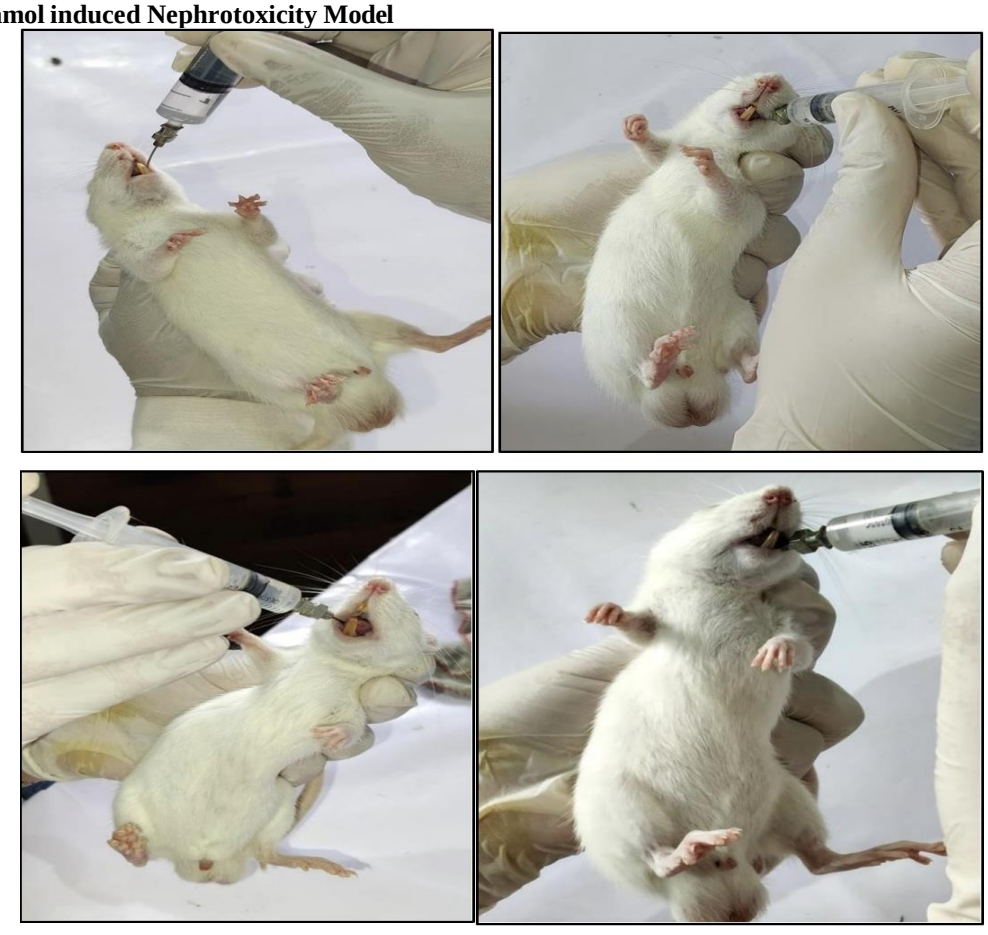


Figure 21: Paracetamol induced Nephrotoxicity Model.

3.5 Measurement of pH, Viscosity determination

Table 17: pH, Viscosity and Spreadability test.

S. No	Formulation	pH	Viscosity determination(cps)	skin irritation study
1.	Formulation1	6.2	1453 ± 0.71 cps	Not irritation observed
2.	Formulation2	6.4	1431 ± 0.25 cps	Not irritation observed
3.	Polyherbal formulation	6.6	1486 ± 0.86 cps	Not irritation observed

***3.6 In vivo* acute oral toxicity (OECD 423)**

Table 18: General appearance and behavioural observations of acute oral toxicity study for control and treated groups.

Parameter	Control	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Food Intake	Normal	Normal	Normal	Normal	Normal
Body Weight Change	Normal	No change	No change	No change	No change
Body Temperature	Normal	Normal	Normal	Normal	Normal
Skin and Fur Condition	No effect	No effect	No effect	No effect	No effect
Urination	Normal	Normal	Normal	Normal	Normal
Diarrhea	Absent	Absent	Absent	Absent	Absent
Mortality	None	None	None	None	None

The acute oral toxicity study, conducted following OECD guideline 423, showed that the test substance was well-tolerated at doses ranging from 5 to 2000 mg/kg. No significant changes were observed in general appearance, behavior, food intake, body weight, or body temperature compared to controls. Skin and fur condition, urination, and gastrointestinal function remained normal, and no mortality occurred at any dose. These results indicate that the polyherbal formulation is safe, with an LD_{50} likely exceeding 2000 mg/kg.

3.7 Analysis of general parameters

3.7.1 Estimation of urine volume

Table 19: Urine volume.

S. No	Groups	Urine Volume (ml)
1	Normal	4.16±1.02
2	Negative Control (750 mg/kg bwt)	8.98±1.05
3	Standard (50 mg/kg)	5.83±0.52
4	Formulation I *Tamarindus indica* suspension	6.99±1.03
5	Formulation II*Pithecellobium dulce*suspension	7.79±0.31
6	Formulation III polyherbal suspension	6.20±1.06

3.7.2 Estimation of Body weight

Table 20: Body weight.

S. No	Groups	Body Weight
1	Normal	252.13±0.260
2	Negative Control (750 mg/kg bwt)	264.15±2.33
3	Standard (50 mg/kg)	255.95±0.249
4	Formulation I *Tamarindus indica* suspension	258.90±0.223
5	Formulation II*Pithecellobium dulce*suspension	260.20±2.06
6	Formulation III polyherbal suspension	257.68±0.289

3.7.3 Estimation of Serum Creatinine

Table 21: Serum Creatinine.

S. No	Groups	Serum Creatinine
1	Normal	0.72±0.06
2	Negative Control (750 mg/kg bwt)	1.30±0.16
3	Standard (50 mg/kg)	0.84±0.17
4	Formulation I *Tamarindus indica* suspension	1.08±0.03
5	Formulation II*Pithecellobium dulce*suspension	0.93±0.20
6	Formulation III polyherbal suspension	0.95±0.19

3.7.4 Estimation of Serum Blood urea nitrogen (BUN)

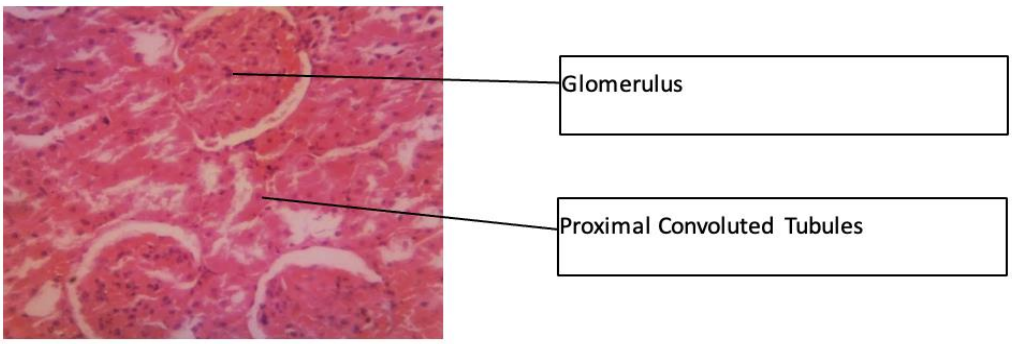
Table 22: Serum Blood urea nitrogen.

S. No	Groups	Serum Blood Urea Nitrogen
1	Normal	25.08±0.53
2	Negative Control (750 mg/kg bwt)	41.11±0.85
3	Standard (50 mg/kg)	27.09±0.54
4	Formulation I *Tamarindus indica* suspension	30.85±0.97
5	Formulation II*Pithecellobium dulce*suspension	28.76±0.56
6	Formulation III polyherbal suspension	31.76±0.98

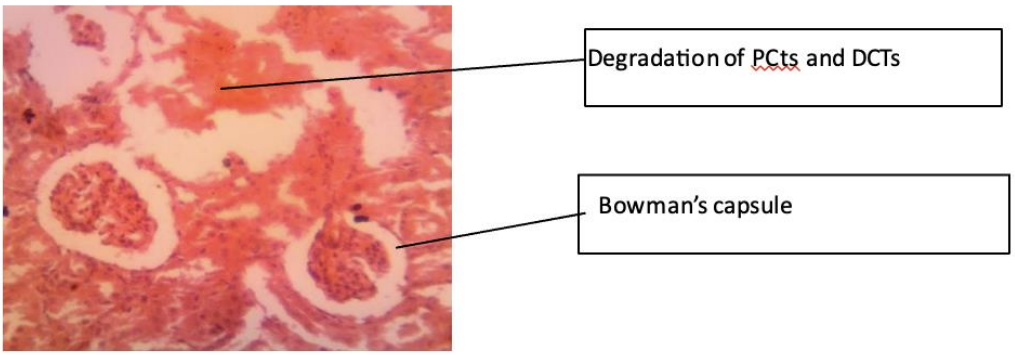
DISCUSSION

Analysis of general parameters highlighted the nephroprotective and diuretic potential of the tested formulations. Urine volume in the negative control group (8.98 ± 1.05 mL) was significantly higher than the normal group (4.16 ± 1.02 mL), indicating impaired renal function or induced diuresis. Among treatments, Formulation II (*Pithecellobium dulce*) showed the highest urine output (7.79 ± 0.31 mL), followed by Formulation I (*Tamarindus indica*) and the polyherbal formulation, all improving over the standard. Minimal body weight changes across groups suggested no systemic toxicity. Serum creatinine was elevated in the negative control (1.30 ± 0.16 mg/dL), while Formulation II (0.93 ± 0.20 mg/dL) and Formulation III (0.95 ± 0.19 mg/dL) approached normal and standard values, indicating improved kidney function. Similarly, BUN levels were high in the negative control (41.11 ± 0.85 mg/dL) and reduced effectively by treatments, with Formulation II (28.76 ± 0.56 mg/dL) closest to the standard (27.09 ± 0.54 mg/dL). Overall, Formulation II demonstrated the most pronounced diuretic and nephroprotective effects, enhancing urine output and reducing serum creatinine and BUN levels relative to the negative control.

Histopathological analysis



GROUP 1



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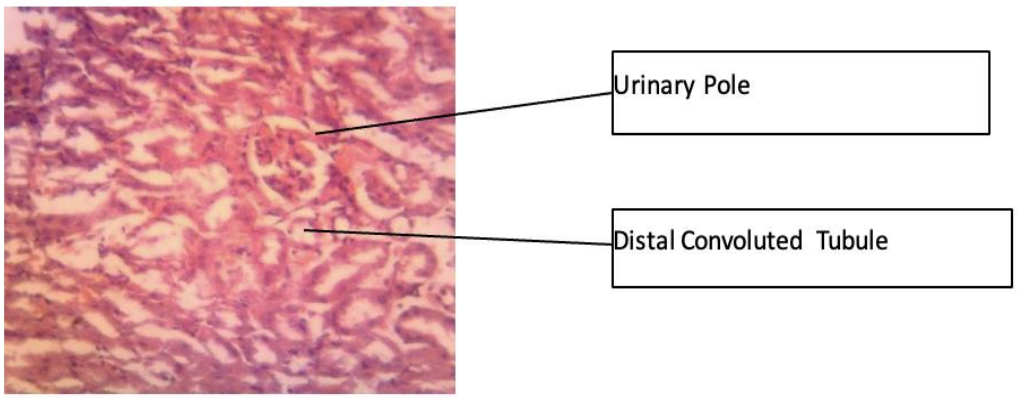

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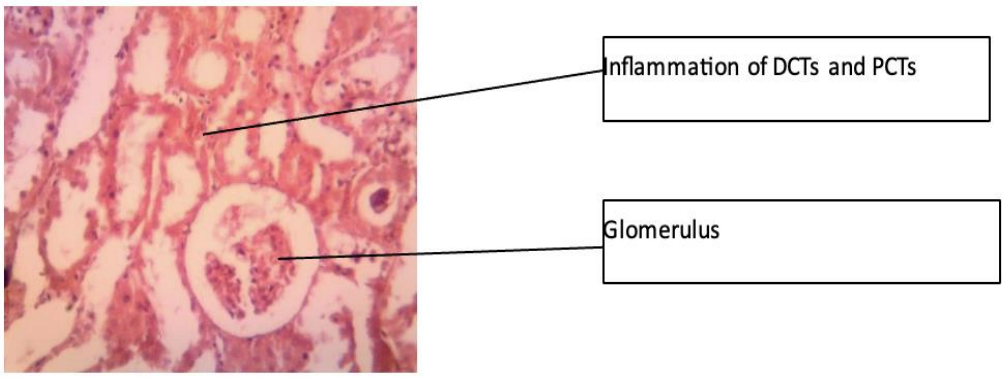
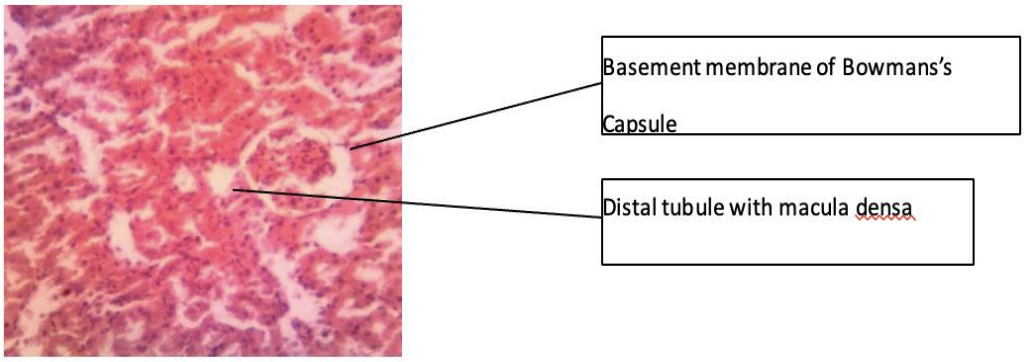


GROUP 4



GROUP 5

Figure 22: Histopathological studies.

DISCUSSION

Histopathological analysis revealed marked differences among groups. The normal group showed intact glomeruli and well-preserved proximal convoluted tubules (PCTs), indicating healthy renal architecture. The negative control exhibited degradation of PCTs and distal convoluted tubules (DCTs) with Bowman's capsule damage, confirming renal injury. The standard-treated group displayed relatively normal structures, with clear urinary poles and DCTs, reflecting protective effects. The *Tamarindus indica* formulation showed mild inflammation in PCTs and DCTs but intact glomeruli, suggesting partial protection. The *Pithecellobium dulce* formulation demonstrated superior preservation, with an intact Bowman's capsule basement membrane and organized distal tubules with macula densa, indicating strong nephroprotection. Overall, histological findings corroborate biochemical results, with *Pithecellobium dulce* showing the most pronounced renal protective effect, followed by *Tamarindus indica* and the standard treatment.

4. CONCLUSION

The present study demonstrated that *Tamarindus indica* and *Pithecellobium dulce*, individually and in combination, possess significant nephroprotective potential. Phytochemical analysis confirmed the presence of diverse bioactive compounds, including phenols, flavonoids, alkaloids, tannins, and glycosides, which likely contribute to their therapeutic effects. Acute toxicity studies established that the extracts and formulations are safe at high doses, with no observable adverse effects. Polyherbal and single-plant suspensions improved renal function in Wistar rats, as evidenced by enhanced urine output and reduced serum creatinine and blood urea nitrogen levels. Histopathological evaluation further corroborated these findings, showing preservation of glomerular and tubular structures, particularly in *Pithecellobium dulce*-treated groups. Overall, the study supports the traditional use of these plants in managing renal disorders and highlights *Pithecellobium dulce* as a particularly effective nephroprotective agent. These results provide a scientific basis for further exploration of these plants in the development of safe and effective herbal therapeutics for kidney health.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest related to the formulation, analysis, or reporting of the data presented in this study.

6. ACKNOWLEDGEMENT

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