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INVESTIGATION OF THE RENO PROTECTIVE EFFECTS OF HERBAL PLANT EXTRACT ON EXPERIMENTALLY INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT
The present study investigated the nephroprotective potential of *Golden leather fern*, a traditionally used mangrove fern with reported medicinal properties. The plant was selected based on ethnobotanical evidence supporting its role in renal and oxidative stress–related disorders. Fresh plant material was collected, authenticated, shade-dried, powdered, and subjected to Soxhlet extraction to obtain bioactive constituents. The percentage yield was calculated to assess extraction efficiency. Preliminary phytochemical screening was performed to identify major classes of bioactive compounds, and quantitative estimation of total phenolic and flavonoid contents was carried out using standard colorimetric assays. The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay. Acute oral toxicity studies were conducted according to OECD guidelines to establish the safety profile and determine appropriate dose levels. Nephroprotective activity was assessed in albino rats with experimentally induced nephrotoxicity. Renal function was evaluated by measuring urine output, body weight changes, serum creatinine, and blood urea nitrogen levels. Histopathological examination of kidney tissues was performed to assess structural and cellular alterations. Statistical analysis of the results demonstrated a significant nephroprotective effect of the plant extract, likely mediated through its antioxidant and phytochemical constituents. The findings support the traditional use of Golden leather fern and suggest its potential as a natural therapeutic agent for the management of renal disorders.

KEYWORDS: Nephroprotection; Golden leather fern; Antioxidant activity; Phytochemical screening; Renal toxicity; Albino rats; Oxidative stress.

INTRODUCTION
The kidneys play a vital role in maintaining physiological homeostasis by regulating fluid and electrolyte balance, eliminating metabolic waste products, and controlling blood pressure. Renal impairment or nephrotoxicity, characterized by structural and functional damage to the kidneys, remains a significant clinical problem worldwide. Nephrotoxicity can be induced by a variety of factors, including exposure to environmental toxins, chemicals, heavy metals, and prolonged use of certain therapeutic agents such as antibiotics, non-steroidal anti-inflammatory drugs, and chemotherapeutic agents. Drug-induced nephrotoxicity is one of the leading causes of acute kidney injury and contributes substantially to morbidity and mortality, especially in hospitalized patients.

Experimental models of nephrotoxicity in animals are widely employed to understand the underlying mechanisms of renal damage and to evaluate potential nephroprotective agents. These models commonly involve the administration of nephrotoxic substances that generate excessive reactive oxygen species (ROS), leading to oxidative stress, inflammation, lipid peroxidation, and apoptosis of renal tubular cells. Oxidative stress–mediated damage is considered a central mechanism in the progression of renal injury, ultimately resulting in impaired glomerular filtration and altered biochemical markers such as elevated serum creatinine, urea, and blood urea nitrogen.

In recent years, increasing attention has been directed toward the use of herbal medicines and plant-derived

bioactive compounds for the prevention and management of renal disorders. Medicinal plants are rich sources of antioxidants, flavonoids, phenolic compounds, alkaloids, and other phytoconstituents that exhibit free radical scavenging, anti-inflammatory, and cytoprotective activities. Several traditional medicinal systems have documented the use of herbal remedies for kidney-related ailments, suggesting their potential reno-protective benefits with fewer adverse effects compared to synthetic drugs.

The present study was designed to investigate the reno-protective effects of a selected herbal plant extract against experimentally induced nephrotoxicity in rats. By evaluating biochemical, antioxidant, and histopathological parameters, this research aims to provide scientific evidence supporting the protective role of herbal therapy in renal damage and to explore its potential as a safer and effective therapeutic strategy for nephrotoxicity.

The present study investigated the nephroprotective potential of **Golden leather fern**, a traditionally used medicinal mangrove fern. The plant was selected based on ethnobotanical and pharmacological evidence supporting its role in renal and oxidative stress–related disorders. Fresh plant material was collected, authenticated, shade-dried, and powdered. Extraction was carried out using the Soxhlet method to obtain bioactive constituents, and the percentage yield was determined. Preliminary phytochemical screening and quantitative estimation of total phenolic and flavonoid contents were performed to assess the antioxidant potential of the extract.

The antioxidant activity was evaluated using in vitro assays such as the DPPH free radical scavenging method. Acute oral toxicity studies were conducted according to OECD guidelines to establish the safety and suitable dose range. Nephroprotective activity was assessed in albino rats with experimentally induced nephrotoxicity using a standard nephrotoxic agent. Animals were treated with different doses of the plant extract, and renal function was evaluated by measuring urine output, body weight changes, serum creatinine, and blood urea nitrogen levels. Histopathological examination of kidney tissues was performed to assess structural alterations and protective effects. The collected data were statistically analyzed to determine the nephroprotective efficacy of the extract.

Extraction of Plant Material by Soxhlet Method

The dried and coarsely powdered plant material of *Golden leather fern* was subjected to Soxhlet extraction for the isolation of bioactive constituents. Petroleum ether followed by methanol was used as extraction solvents based on phytochemical polarity. The powdered material was packed in a thimble and placed in the Soxhlet apparatus. Continuous extraction was carried out for 6–8 hours until the siphon tube solvent became colorless, indicating complete exhaustion of the plant material. The obtained extract was filtered and concentrated under reduced pressure using a rotary evaporator. The semi-solid extract was dried in a desiccator and stored in airtight containers at low temperature for further studies. Percentage yield was calculated using the formula:

$$\%\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

Preliminary Phytochemical Screening

The methanolic extract was subjected to qualitative phytochemical analysis to detect carbohydrates, saponins, tannins, phenolics, triterpenoids, steroids, glycosides, proteins, amino acids, alkaloids, and flavonoids using standard chemical tests such as Molisch's, Fehling's, Froth, Ferric chloride, Liebermann–Burchard, Borntrager's, Biuret, Dragendorff's, and alkaline reagent tests.

Quantitative Estimation of Phytoconstituents

Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu method. Absorbance was measured at 760 nm, and results were expressed as mg gallic acid equivalents per gram of extract.

Total Flavonoid Content

Flavonoid content was estimated by the aluminum chloride colorimetric method. Absorbance was measured at 510 nm, and results were expressed as mg rutin equivalents per gram of extract.

Antioxidant Activity (DPPH Assay)

Antioxidant activity was evaluated using the DPPH free radical scavenging assay. Various concentrations (20–100 µg/mL) of the extract were reacted with DPPH solution, and absorbance was measured at 517 nm. Percentage inhibition was calculated using:

$$\%\text{Scavenging Activity} = 100\left(\frac{A_c - A_s}{A_c}\right)$$

Acute Toxicity Study

Acute oral toxicity was evaluated according to OECD guideline 423. No mortality or behavioral abnormalities were observed up to 2000 mg/kg, indicating the extract was safe.

In Vivo Nephroprotective Activity

Adult Wistar albino rats were divided into five groups (n=6). Nephrotoxicity was induced using paracetamol (750 mg/kg). Treatment groups received N-acetylcysteine (50 mg/kg) or *Golden leather fern* extract (250 and 500 mg/kg) for 30 days.

Biochemical and Physiological Analysis

Urine volume, serum creatinine, and blood urea nitrogen (BUN) were estimated using standard diagnostic kits. Body weight changes were recorded weekly. Renal

function was assessed based on biochemical and histopathological parameters.

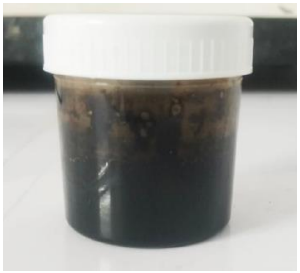


Figure 1: Percentage Yield of plant material.

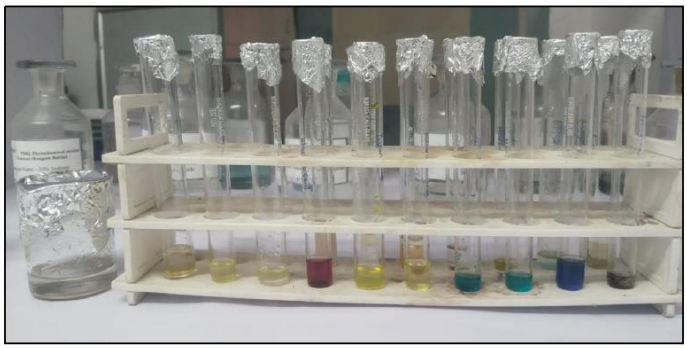


Figure 2: Phytochemical test of Petroleum ether extract.

1.	**Alkaloids**	
1.1	Dragendroff's test	+ ve
1.2	Mayer's reagent test	+ ve
1.3	Wagner's reagent test	+ ve
1.3	Hager's reagent test	+ ve
2.	**Glycoside**	
2.1	Borntrager test	+ ve
2.2	Legal's test	+ ve
2.3	Killer-Killiani test	+ ve
3.	**Carbohydrates**	
3.1	Molish's test	- ve
3.2	Fehling's test	- ve
3.3	Benedict's test	- ve
3.4	Barfoed's test	- ve
4.	**Proteins and Amino Acids**	
4.1	Biuret test	- ve
4.2	Ninhydrin test	- ve
5.	**Flavonoids**	
5.1	Alkaline reagent test	+ ve
5.2	Lead Acetate test	+ ve
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	+ ve
7.	**Saponin**	
7.1	Foam test	+ ve
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	- ve
8.2	Libbermann-Burchard's test	- ve

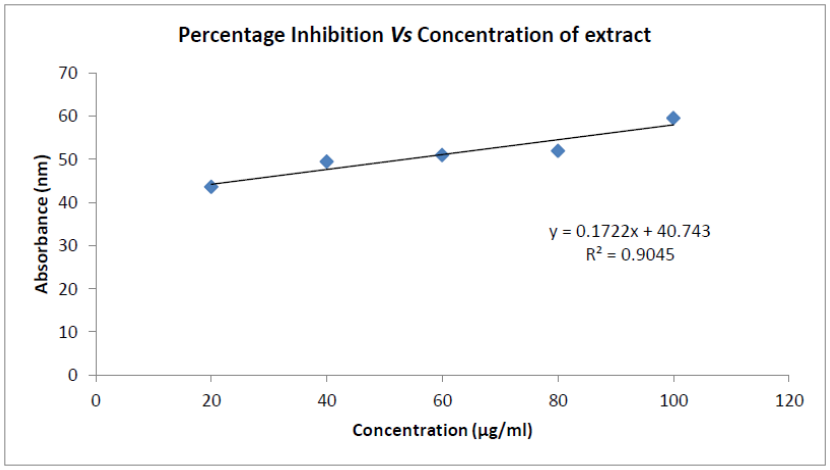


Figure 3: Represents the Percentage Inhibition *Vs* Concentration of *Golden leather fern* extract.

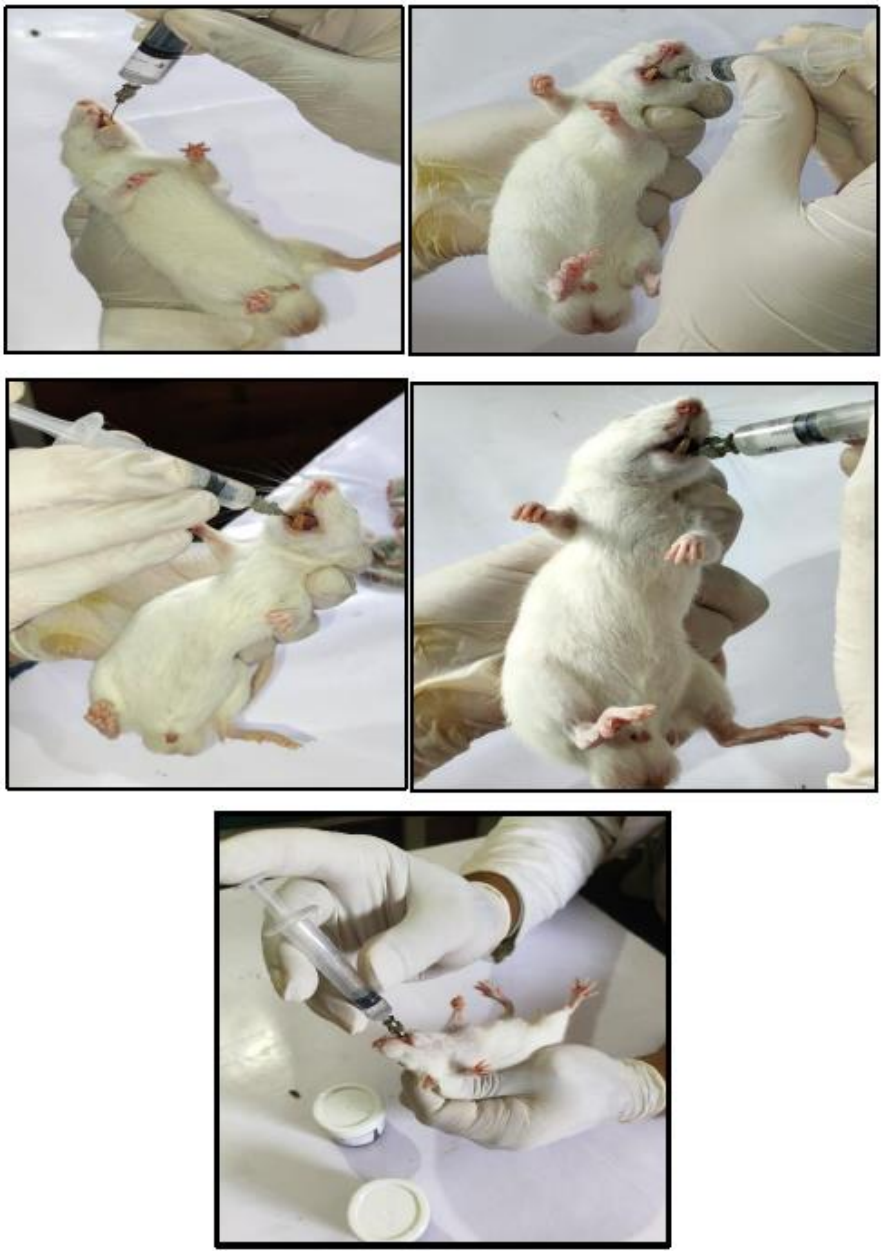


Figure 4: Paracetamol induced Nephrotoxicity Model.

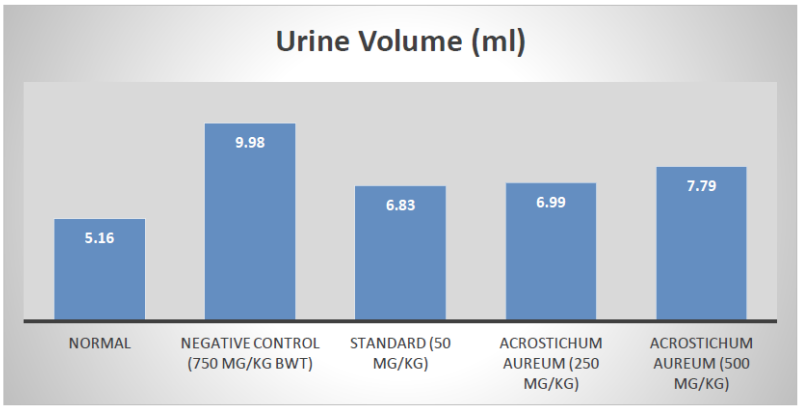


Figure 5: Urine volume.

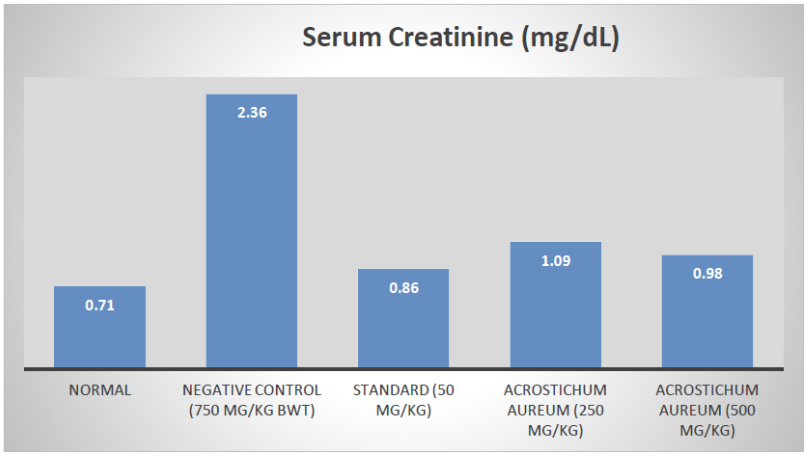
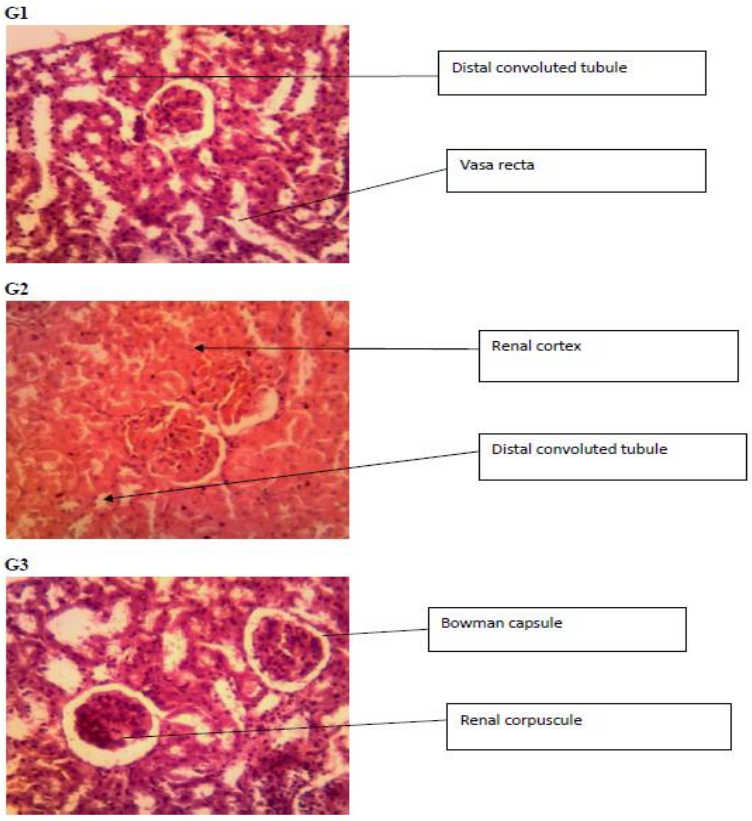


Figure 6: Serum Creatinine.

Histopathological studies



Histopathological examination of kidney tissues revealed distinct structural differences across the groups. In the normal control group (G1 and G2), the renal architecture appeared intact, with clearly defined distal convoluted tubules, vasa recta, and renal cortex, indicating healthy kidney function. In the negative control group (G3), signs of renal damage were evident, including distortion in Bowman's capsule and renal corpuscle structure, suggesting glomerular injury.

Group G4 showed congested blood vessels and changes in the glomerulus, reflecting possible inflammation or vascular compromise. However, in the treated group (G5), the collecting tubules and renal artery appeared more preserved, indicating partial restoration of normal histology. These observations support the biochemical findings, suggesting that the *Golden leather fern* extract may exert a protective effect on renal tissue architecture, particularly at higher doses.

The present study evaluated the nephroprotective potential of *Golden leather fern (A. aureum)* through extraction yield analysis, phytochemical screening, antioxidant assessment, and an in vivo paracetamol-induced nephrotoxicity model. Methanolic extraction yielded a higher percentage (5.60%) compared to petroleum ether (4.67%), indicating methanol as a more efficient solvent for extracting bioactive constituents. Phytochemical screening revealed that the methanolic extract was rich in alkaloids, glycosides, flavonoids, tannins, and saponins, whereas the petroleum ether extract lacked alkaloids and glycosides. Quantitative analysis showed appreciable total phenolic content (76 mg GAE/g) and total flavonoid content (28.1 mg RE/g), suggesting strong antioxidant potential.

The methanolic extract exhibited notable antioxidant activity in the DPPH assay, with an IC_{50} value of 53.81 µg/mL, supporting its role in mitigating oxidative stress. In vivo studies demonstrated dose-dependent nephroprotection, as evidenced by normalization of urine volume, body weight, serum creatinine, and blood urea nitrogen levels in treated groups. The higher dose (500 mg/kg) showed effects comparable to the standard drug. Histopathological examination further confirmed renal protection, showing preserved renal architecture and reduced tissue damage in extract-treated groups. Overall, the findings indicate that *A. aureum* possesses significant nephroprotective activity, likely mediated through its antioxidant and phytochemical constituents.

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