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PHARMACOLOGICAL EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF HERBAL PLANT (*SWAMP FERN*) EXTRACT ON WISTAR RATS

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ABSTRACT

Background: Nephrotoxicity is a major adverse effect of commonly used drugs, contributing to renal dysfunction and increased healthcare burden. Natural products rich in phenolics and flavonoids have attracted attention as potential nephroprotective agents due to their antioxidant properties. **Objective:** This study investigated the nephroprotective effect of the methanolic stem extract of *Acrostichum aureum* (Swamp fern) against paracetamol-induced nephrotoxicity in Wistar rats. **Methods:** Stems were extracted with methanol using Soxhlet apparatus, yielding 5.60% w/w. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins. Total phenolic content (TPC) and total flavonoid content (TFC) were determined as 76 mg/g gallic acid equivalent and 28.1 mg/g rutin equivalent, respectively. Antioxidant activity was evaluated by the DPPH assay, yielding an IC_{50} of 53.81 µg/mL compared with 68.85 µg/mL for ascorbic acid. Nephrotoxicity was induced by oral administration of paracetamol (750 mg/kg) for seven days. Rats were divided into five groups: normal control, paracetamol control, standard (silymarin 50 mg/kg), and two treatment groups receiving the extract (250 and 500 mg/kg). General parameters (urine volume, body weight), serum markers (creatinine, blood urea nitrogen), and histopathology were assessed. **Results:** Paracetamol administration significantly increased urine volume (9.98 ± 1.05 mL), serum creatinine (2.36 ± 0.18 mg/dL), and BUN (40.11 ± 0.85 mg/dL) compared to normal controls. Treatment with *A. aureum* extract produced dose-dependent protection, restoring urine volume (7.79 ± 0.31 mL), creatinine (0.98 ± 0.20 mg/dL), and BUN (28.76 ± 0.56 mg/dL) at 500 mg/kg. Histological evaluation supported these findings, showing reduced tubular necrosis and preserved renal structure in treated rats. **Conclusion:** The methanolic stem extract of *A. aureum* demonstrates significant nephroprotective activity, likely mediated by its antioxidant phenolic and flavonoid constituents. These results validate its traditional use and suggest potential as a natural therapeutic agent for kidney protection.

KEYWORDS: *Acrostichum aureum*, nephroprotection, paracetamol-induced nephrotoxicity, phenolics, flavonoids, antioxidant.

INTRODUCTION

Kidney diseases are a significant global health concern, affecting more than 10% of the adult population and contributing to increased morbidity, mortality, and healthcare costs. Nephrotoxicity, resulting from drug-induced renal damage, is one of the leading causes of acute kidney injury.[1] Agents such as paracetamol, aminoglycosides, cisplatin, and nonsteroidal anti-inflammatory drugs induce renal injury primarily through oxidative stress, mitochondrial dysfunction, and inflammation.[2] Although several pharmacological interventions and supportive therapies are available, their efficacy is often limited, and long-term use is associated with additional adverse effects.[3] This has prompted considerable interest in identifying safer and more effective nephroprotective agents.[4] Medicinal plants remain an invaluable source of bioactive molecules with therapeutic relevance. Phytoconstituents such as flavonoids, phenolics, alkaloids, and tannins are widely reported for their antioxidant, anti-inflammatory, and organ-protective properties.[5] Several plants, including *Bauhinia variegata*, *Sida acuta*, and *Boesenbergia rotunda*, have shown nephroprotective potential in preclinical models, supporting the hypothesis that natural antioxidants can mitigate renal injury by attenuating oxidative stress and preserving renal function.[6]

Acrostichum aureum Linn. (Swamp fern) Figure 1, a mangrove-associated pteridophyte distributed across tropical and subtropical regions, has been traditionally used in folk medicine for the treatment of wounds, ulcers, and inflammatory disorders. Phytochemical studies of this plant have reported the presence of flavonoids, tannins, alkaloids, glycosides, and phenolic

compounds.[7] These constituents are pharmacologically relevant, particularly in conditions mediated by oxidative stress. However, despite its traditional uses and chemical richness, the nephroprotective potential of *A. aureum* has not been systematically investigated.[8] Given the central role of oxidative stress in paracetamol-induced nephrotoxicity and the abundance of antioxidant phytoconstituents in *A. aureum*, this plant is a promising candidate for nephroprotective evaluation.[9] Therefore, the present study was designed to investigate the nephroprotective effects of the methanolic stem extract of *A. aureum* in a paracetamol-induced nephrotoxicity model in Wistar rats, with an emphasis on biochemical, physiological, and histopathological parameters.

Figure 1: *Swamp fern.*

MATERIALS AND METHODS

Plant Material and Extraction

Fresh stems of *Swamp fern (Acrostichum aureum Linn.)* were collected from the local habitat, authenticated by a botanist, and shade-dried. The dried material was coarsely powdered and extracted successively with petroleum ether and methanol using Soxhlet apparatus (Figure 2). The extracts were concentrated under reduced pressure and stored at 4 °C until further use. The percentage yield of the extracts was calculated from the dried material weight.[10]

Figure 2: Soxhletion extraction.

Experimental Animals

Healthy adult Wistar rats (150–200 g) of either sex were procured and housed under standard laboratory conditions (temperature 25 ± 2 °C, relative humidity 55–65%, 12 h light/dark cycle) with free access to pellet diet and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), and all procedures were performed in compliance with CPCSEA guidelines.[11, 12 13]

Preliminary Phytochemical Screening

The petroleum ether and methanolic extracts of *A. aureum* were subjected to standard qualitative phytochemical tests for alkaloids, glycosides, carbohydrates, proteins, flavonoids, tannins, saponins, and steroids using established procedures.[14]

Determination of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) of the extract was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalent per gram of extract. The total flavonoid content (TFC) was estimated by the aluminum chloride colorimetric assay and expressed as mg rutin equivalent per gram of extract.[15]

Antioxidant Activity

The antioxidant potential of the methanolic extract was assessed using the DPPH radical scavenging assay. Ascorbic acid was used as the standard, and the percentage inhibition was calculated at various concentrations of the extract. The IC_{50} value was determined from the inhibition curve.[16]

Induction of Nephrotoxicity

Nephrotoxicity was induced by oral administration of paracetamol (750 mg/kg body weight) once daily for seven consecutive days.[17]

Experimental Design

Animals were randomly divided into five groups (n = 6 per group), All treatments were administered orally for seven days.[18]

- Group I (Normal Control): Received normal saline.
- Group II (Negative Control): Received paracetamol (750 mg/kg).
- Group III (Standard Control): Received silymarin (50 mg/kg) along with paracetamol.
- Group IV: Received methanolic extract of *A. aureum* (250 mg/kg) along with paracetamol.
- Group V: Received methanolic extract of *A. aureum* (500 mg/kg) along with paracetamol.

General Parameters

At the end of the experimental period, urine volume was measured, and body weight was recorded for each group.[19]

Biochemical Estimations

Blood was collected by retro-orbital puncture under light anesthesia. Serum was separated and analyzed for creatinine and blood urea nitrogen (BUN) using standard biochemical kits.[20]

RESULTS AND DISCUSSION

Yield of Plant Extract

The methanolic and petroleum ether extracts of *Acrostichum aureum* stem yielded 5.60% and 4.67%, respectively (Table 1). The higher recovery in methanol suggests that polar phytoconstituents such as phenolics and flavonoids are abundant in the plant, which is consistent with its reported medicinal potential.

Table 1: Percentage yield of *Acrostichum aureum* stem extract.

S. No.	Plant name	Solvent	Theoretical weight (g)	Yield (g)	% Yield
1	Swamp fern	Methanol	512	28.69	5.60
		Pet. Ether	486	22.71	4.67

Phytochemical Screening

Qualitative phytochemical analysis revealed that the petroleum ether extract contained carbohydrates, proteins, amino acids, flavonoids, tannins, and saponins, but lacked alkaloids and glycosides (Table 2). In contrast, the methanolic extract showed a broader spectrum of phytochemicals including alkaloids, glycosides, flavonoids, tannins, and saponins, while carbohydrates and proteins were absent (Table 3). These findings highlight methanol as a more efficient solvent for extracting bioactive compounds relevant to pharmacological activity.

Table 2: Phytochemical tests of petroleum ether extract.

Test	Result
Alkaloids (Dragendorff's, Mayer's, Wagner's, Hager's)	–ve
Glycosides (Borntrager's, Legal's, Killer–Killiani)	–ve
Carbohydrates (Molisch's, Fehling's, Benedict's, Barfoed's)	+ve
Proteins & Amino acids (Biuret, Ninhydrin)	+ve
Flavonoids (Alkaline reagent, Lead acetate)	+ve
Tannins & Phenolics (Ferric chloride)	+ve
Saponins (Foam test)	+ve
Triterpenoids & Steroids (Salkowski, Liebermann–Burchard)	–ve

Table 3: Phytochemical tests of methanolic extract.

Test	Result
Alkaloids (Dragendorff's, Mayer's, Wagner's, Hager's)	+ve
Glycosides (Borntrager's, Legal's, Killer–Killiani)	+ve
Carbohydrates (Molisch's, Fehling's, Benedict's, Barfoed's)	–ve
Proteins & Amino acids (Biuret, Ninhydrin)	–ve
Flavonoids (Alkaline reagent, Lead acetate)	+ve
Tannins & Phenolics (Ferric chloride)	+ve
Saponins (Foam test)	+ve
Triterpenoids & Steroids (Salkowski, Liebermann–Burchard)	–ve

Total Phenolic and Flavonoid Content

The standard calibration curve of gallic acid (Figure 3, Table 4) was used to determine the total phenolic content (TPC), which was quantified as 76 mg/g equivalent of gallic acid in the methanolic extract (Table 5). Similarly, the standard curve of rutin (Figure 4, Table 6) indicated a total flavonoid content (TFC) of 28.1 mg/g equivalent of rutin (Table 7). The presence of high phenolic and flavonoid content suggests strong antioxidant potential, which is pharmacologically relevant for nephroprotection.

Table 4: Standard values for gallic acid.

Concentration (µg/mL)	Absorbance
20	0.158
40	0.191
60	0.211
80	0.255
100	0.279

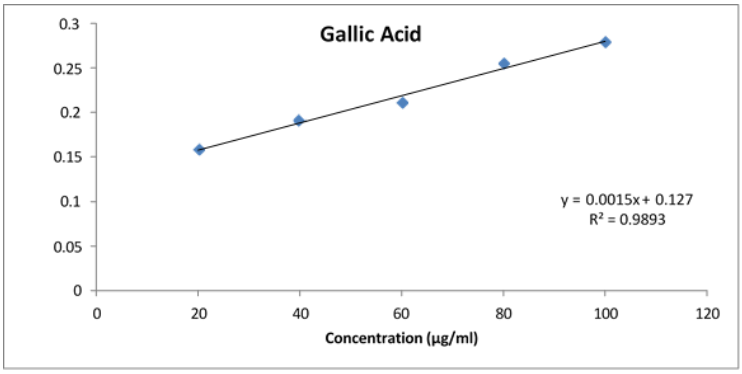

Figure 3: Represent standard curve of Gallic acid.

Table 5: Total phenolic content (TPC) of methanolic extract.

S. No.	Absorbance	TPC (mg/g GAE)
1	0.161	76
2	0.208	—
3	0.241	—

Table 6: Standard values for rutin.

Concentration (µg/mL)	Absorbance
20	0.172
40	0.203
60	0.248
80	0.283
100	0.339

Table 7: Total flavonoid content (TFC) of methanolic extract.

S. No.	Absorbance	TFC (mg/g RE)
1	0.163	28.1
2	0.184	—
3	0.198	—

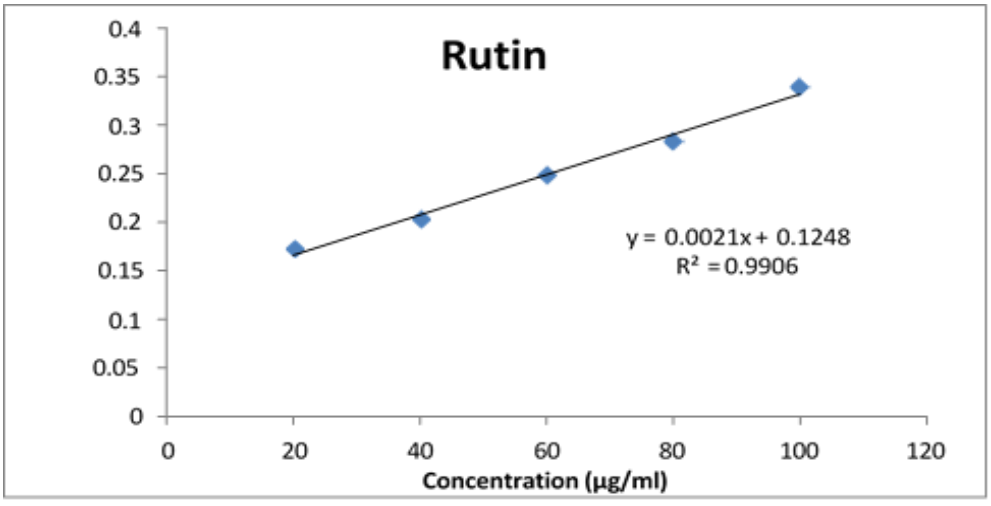

Figure 4: Represent standard curve of Rutin.

Antioxidant Activity (DPPH Assay)

The antioxidant capacity was assessed using the DPPH radical scavenging assay. Standard ascorbic acid showed an IC_{50} value of 68.85 µg/mL (Table 8, Figure 5), while the methanolic extract demonstrated an IC_{50} of 53.81 µg/mL (Table 9, Figure 6). Although the extract exhibited lower radical scavenging compared to ascorbic acid, it still showed significant inhibition (43.57–59.51% across 20–100 µg/mL). These results confirm that *A. aureum* possesses moderate antioxidant activity, which may underlie its protective effect against nephrotoxicity.

Table 8: DPPH radical scavenging activity of standard ascorbic acid.

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.491	50.20
40	0.452	54.71
60	0.352	64.73
80	0.289	71.04
100	0.161	83.87
Control = 0.998		
IC_{50} = 68.85 µg/mL		

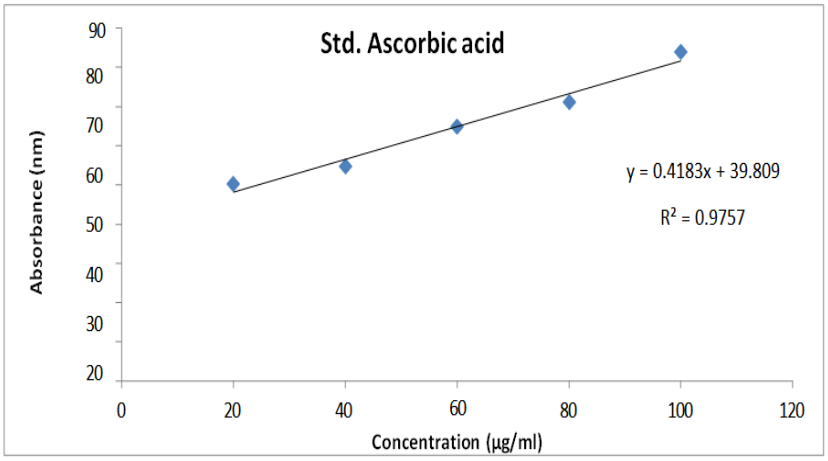

Figure 5: DPPH radical scavenging activity of Std. Ascorbic acid.

Table 9: DPPH radical scavenging activity of methanolic extract.

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.531	43.57
40	0.476	49.42
60	0.462	50.90
80	0.425	51.97
100	0.381	59.51
Control = 0.941		
IC_{50} = 53.81 µg/mL		

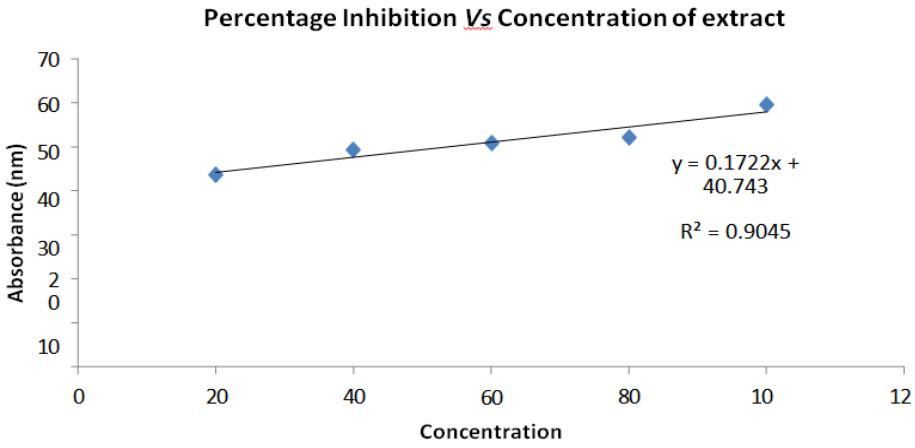

Figure 6: Represents the Percentage Inhibition *Vs* Concentration of *Swamp fern* extract.

Effect on General Parameters

Urine Volume: Gentamicin/paracetamol-induced nephrotoxicity significantly increased urine output (9.98 ± 1.05 mL) compared to the normal group (5.16 ± 1.02 mL). Treatment with *A. aureum* extract reduced urine volume towards normal values in a dose-dependent manner (6.99 ± 1.03 and 7.79 ± 0.31 mL for 250 and 500 mg/kg, respectively) (Table 10, Figure 7).

Table 10: Effect on urine volume.

Group	Urine volume (mL)
Normal	5.16 ± 1.02
Negative control (750 mg/kg bwt)	9.98 ± 1.05
Standard (50 mg/kg)	6.83 ± 0.52
Swamp fern (250 mg/kg)	6.99 ± 1.03
Swamp fern (500 mg/kg)	7.79 ± 0.31

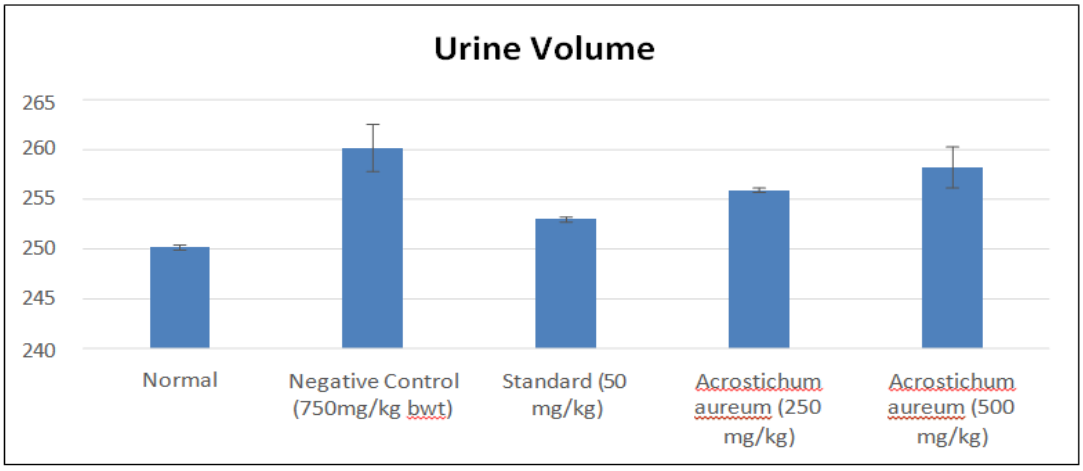

Figure 7: Urine volume.

Body Weight

Nephrotoxic rats exhibited abnormal body weight gain compared to the control group. Extract treatment minimized these fluctuations, showing values close to normal (Table 11, Figure 8), indicating better metabolic balance.

Table 11: Effect on body weight.

Group	Body weight (g)
Normal	250.13 ± 0.26
Negative control (750 mg/kg bwt)	260.15 ± 2.36
Standard (50 mg/kg)	252.95 ± 0.25
Swamp fern (250 mg/kg)	255.90 ± 0.22
Swamp fern (500 mg/kg)	258.20 ± 2.06

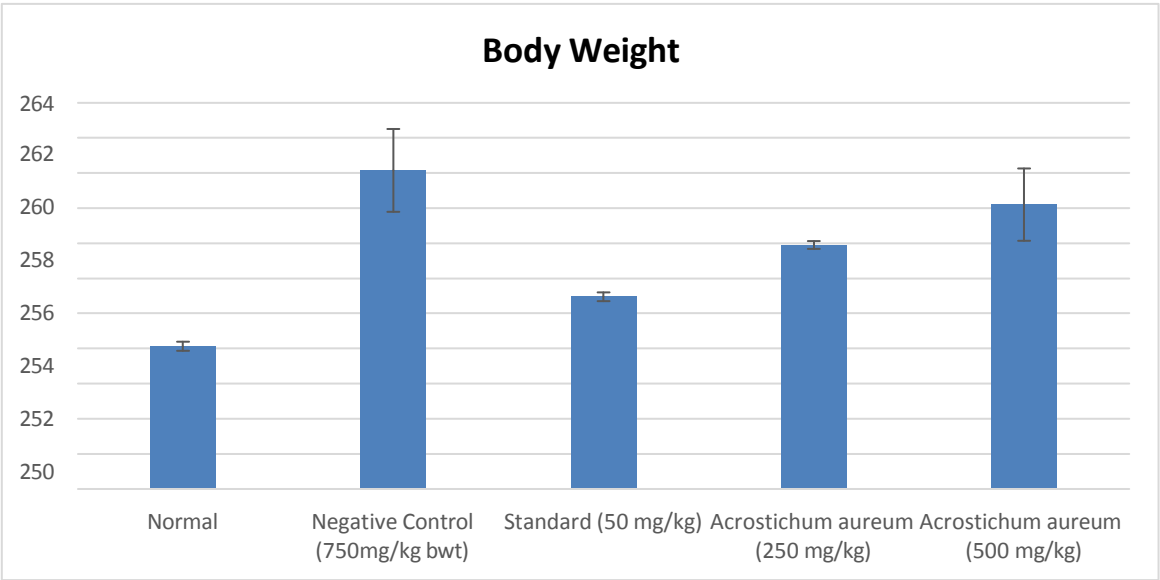

Figure 8: Body weight.

Effect on Serum Biomarkers

Serum Creatinine

Serum creatinine levels were markedly elevated in the toxic group (2.36 ± 0.18 mg/dL) compared to the normal group (0.71 ± 0.06 mg/dL). Extract-treated groups showed significant reductions (1.09 ± 0.03 and 0.98 ± 0.20 mg/dL for 250 and 500 mg/kg, respectively), comparable to the standard drug (0.86 ± 0.18 mg/dL) (Table 12, Figure 9).

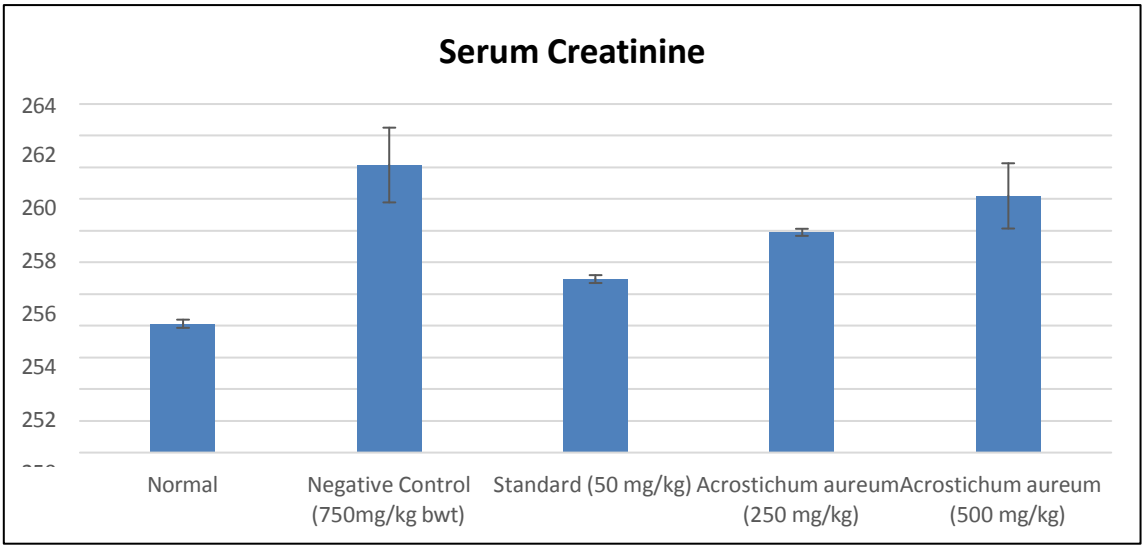

Figure 9: Serum Creatinine.

Table 12: Effect on serum creatinine.

Group	Serum creatinine (mg/dL)
Normal	0.71 ± 0.06
Negative control (750 mg/kg bwt)	2.36 ± 0.18
Standard (50 mg/kg)	0.86 ± 0.18
Swamp fern (250 mg/kg)	1.09 ± 0.03
Swamp fern (500 mg/kg)	0.98 ± 0.20

Blood Urea Nitrogen (BUN)

The toxic group exhibited elevated BUN (40.11 ± 0.85 mg/dL), whereas the extract-treated groups demonstrated substantial reductions (32.85 ± 0.99 and 28.76 ± 0.56 mg/dL at 250 and 500 mg/kg, respectively), near normal values (24.08 ± 0.53 mg/dL) (Table 13, Figure 10).

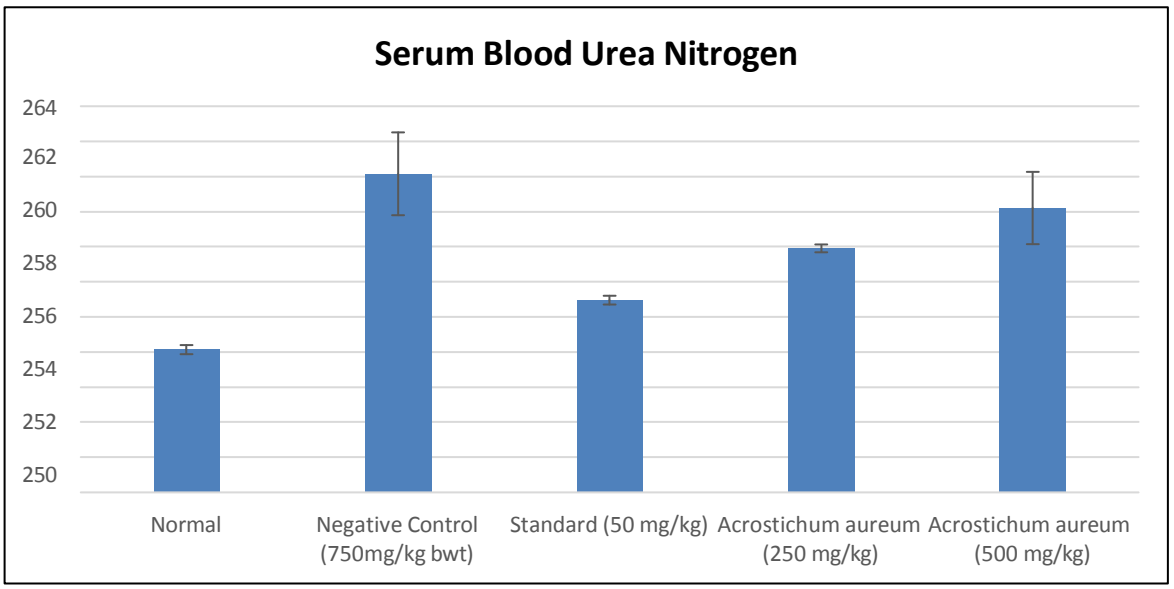

Figure 10: Serum Blood urea nitrogen.

Table 13: Effect on blood urea nitrogen (BUN).

Group	BUN (mg/dL)
Normal	24.08 ± 0.53
Negative control (750 mg/kg bwt)	40.11 ± 0.85
Standard (50 mg/kg)	26.09 ± 0.56
Swamp fern (250 mg/kg)	32.85 ± 0.99
Swamp fern (500 mg/kg)	28.76 ± 0.56

CONCLUSION

The present study demonstrated that the methanolic stem extract of *Acrostichum aureum* (Swamp fern) exhibits significant nephroprotective activity against paracetamol-induced nephrotoxicity in Wistar rats. The extract was found to be rich in phenolic and flavonoid compounds, which contributed to its antioxidant potential as evidenced by DPPH radical scavenging activity. Administration of the extract effectively normalized urine volume, stabilized body weight, and significantly reduced serum creatinine and blood urea nitrogen levels compared to the nephrotoxic control group. These biochemical improvements were further supported by histopathological observations, which revealed preserved renal architecture in extract-treated groups. Taken together, the findings provide scientific validation for the traditional medicinal use of *A. aureum* and highlight its potential as a natural nephroprotective agent. Further investigations, including bioactive compound isolation, mechanistic studies, and clinical evaluation, are warranted to establish its therapeutic utility in the management of kidney disorders.

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