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INVESTIGATION OF THE NEPHROPROTECTIVE ACTIVITY OF *SIDA ACUTA* ETHANOLIC EXTRACT IN RATS

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ABSTRACT
The phytochemical profile and nephroprotective effects of the ethanolic extract of *Sida acuta* were evaluated in this study. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, diterpenes, phenols, proteins, saponins, carbohydrates, tannins, and sterols in varying degrees across different extracts. The ethanolic extract exhibited the broadest spectrum of bioactive compounds. To assess nephroprotective potential, the ethanolic extract was tested in a cisplatin-induced nephrotoxicity model in Wistar rats. The extract significantly improved biochemical markers of kidney function, including serum creatinine, serum urea, blood urea nitrogen (BUN), and oxidative stress indicators such as superoxide dismutase (SOD) and reduced glutathione (GSH). Additionally, it reduced lipid peroxidation and inflammatory markers, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). These findings suggest that the ethanolic extract of *Sida acuta* possesses considerable nephroprotective and anti-inflammatory properties, supporting its potential therapeutic application in preventing and mitigating renal damage.

Keywords: *Sida acuta*, Phytochemical Screening, Nephroprotective Effects, Cisplatin-Induced Nephrotoxicity, Bioactive Compounds, Oxidative Stress, Inflammatory Markers.

INTRODUCTION
According to emerging evidence, nephrotoxicity is one of the most persistent kidney problems with an 8–15% lifetime risk in Europe, 2–5% in Asia, and 20% in the Middle East (Yadav *et al.,* 2017). Nephrotoxicity leads to a reduction in the glomerular filtration rate and an increase in creatinine and blood urea nitrogen in the serum, ultimately increasing the blood pressure and fluid retention in the body (over-hydration). Kidneys are the primary target organ to bear toxic effects of medication. Kidneys account for 25% of the heat output and are naturally exposed to circulatory drugs and chemicals as central excretion bodies. These nephrotoxic drugs contribute to acute kidney failure and increased morbidity and death (Muller *et al.,* 2017; Shah *et al.,* 2020). Because of their functions in glomerular concentrations, drug delivery, and metabolism, the epithelial cells of the renal proximal convoluted tubules (PCT) are a crucial target for nephrotoxicants. Cisplatin (Cis) is the most commonly used potential chemotherapeutic agent against different solid tumors, including those in the head, neck, lung, breast, bladder, and ovary (Shi *et al.,* 2018).
Nephrotoxicity is the most common adverse effect of Cis accumulation in kidneys after

chemotherapy. The Cis disturbs the equilibrium between antioxidants and peroxides, while renal fibrosis is closely related to a rise in oxidative damage (Abdel-Daim *et al.,* 2019). The Cis-complex moves through the cell membranes in a unionized form due to its high chloride concentration in the plasma. Cl-plasma is higher than the intracellular concentration, and chloride ligands are displaced by water, resulting in a nephrotoxic formation of the positive platinum complexes. The Cis molecule binds to the guanine DNA base and inhibits DNA, RNA, and protein synthesis. Cis binds to the DNA interface, and an intrastrand is established, leading to a faulty genetic code model and the arrest of the formation and duplication of DNA replication (Fang *et al.,* 2021).

During the past few decades, natural compounds have been considered among the promising therapeutic agents against cancer, cardiovascular diseases, aging, diabetes, and especially neurodegenerative disorders due to their wide variety of modes of action, efficiency, accuracy, and fewer side effects (Cayir *et al.,* 2011). Several studies have focused currently on traditional herbal medicines to evaluate novel therapeutic drugs for acute kidney injury (AKI) therapy.

Sida acuta has been used in various indigenous medicinal systems, particularly in Ayurveda and traditional African medicine, for its therapeutic potential in treating ailments such as fever, asthma, inflammation, and as a tonic to strengthen the body (Cao and Qi, 1993). The plant is known for its diverse pharmacological properties, attributed to its rich phytochemical composition, including alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. These compounds contribute to its various pharmacological activities. The current nephroprotective study serves as a necessary basis for further studies developing herbal medicine from *Sida acuta.*

MATERIALS AND METHODS

Materials

Ethanol, Chloroform, Ethyl Acetate, Lead Acetate, Ferric Chloride, Dragendroff's Reagent, Molisch's Reagent, Potassium Mercuric Iodide, Potassium Iodide, Iodine, obtained from SRL (S.D. Fine-Chem Ltd.) Mumbai, India. Hydrochloric acid, methanol and ethanol were obtained from Merck Ltd, Mumbai, India.

Collection and extraction of plant materials

Leaves of *Sida acuta* were sourced from Shubham Nursery, Bhopal. Seventy-five grams of shade-dried plant material was coarsely powdered and extracted using petroleum ether in a Soxhlet apparatus until complete defatting of the material was achieved. The defatted, air-dried, and powdered marc of *Sida acuta* was then subjected to sequential extraction with chloroform, ethyl acetate, ethanol, and water, also using a Soxhlet apparatus (Harborne, 1981). After extraction, the mixtures were allowed to stand for at least 48 hours. The resulting extracts were filtered using Whatman filter paper no. 1, and the solvents were evaporated to obtain dry, concentrated extracts.

Phytochemical analysis

The qualitative phytochemical screening of the extracts was carried out using standard methods to identify the presence of various bioactive compounds (Trease and Evans, 1989). The screening involved testing for alkaloids, glycosides, flavonoids, saponins, tannins, phenols, diterpenes, proteins, carbohydrates, sterols, and lignins.

Experimental design - Nephroprotective activity

The in-vivo nephrotoxicity study was conducted following approval from the Institutional Animal Ethics Committee (IAEC), established under the guidelines for the control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi. Wistar rats, weighing between 150 and 200 g, were selected for the study. The animals underwent a 1-week acclimatization period, during which they were maintained at a controlled temperature of 22 ± 1°C and humidity levels of 50–80%. A 12-hour light-dark cycle was followed, and the animals had free access to a standard rodent diet and pre-filtered water.

The ethanolic extract of *Sida acuta* was evaluated for acute oral toxicity in compliance with OECD guidelines ANNEX-423. Based on previous toxicity studies, the ethanolic extract was administered orally to Wistar rats at a dose of 2000 mg/kg body weight. The animals were closely observed for signs of toxicity and adverse effects to ensure the safety and viability of the extract for subsequent testing.

The experimental study was divided into seven groups to assess the nephroprotective effects of the ethanolic extracts of *Sida acuta* (Sultana *et al.,* 2012). Group I served as the control group and received a daily oral dose of the vehicle (saline). Group II, the CP group, was administered cisplatin (CP) injections at a dose of 3 mg/kg/day intraperitoneally (i.p.) every five days to induce nephrotoxicity. Group III consisted of CP-induced nephrotoxic rats treated with *Sida acuta* ethanolic leaf extract at a dose of 100 mg/kg orally per day, and Group IV received a higher dose of *Sida acuta* extract at 200 mg/kg orally per day. Group V, the standard group, received Gentamycin at a dose of 5 mg/kg per day as a reference treatment. At the end of the study, all animals were sacrificed, and biochemical assessments were performed to evaluate the nephroprotective potential of the extracts.

Blood samples were screened to determine various biochemical parameters. Twenty-four hours after the last treatment, blood samples were collected from the animals via retro-orbital puncture. The blood samples were allowed to clot, and serum was rapidly separated by centrifugation. The collected serum was then analyzed for biochemical parameters including serum creatinine, serum urea, serum uric acid, and blood urea nitrogen (BUN) (Farooqui *et al.,* 2017). These markers were assessed as indicators of kidney damage using commercially available diagnostic kits from Span Diagnostics Private Ltd. The results from these analyses were used to determine the extent of nephrotoxicity and evaluate the potential protective effects of the plant extract.

RESULTS AND DISCUSSION

The phytochemical screening of *Sida acuta* extracts reveals a varied profile of chemical constituents across different solvent extracts. Alkaloids were detected only in the ethanolic extract, as indicated by positive results in Wagner's and Hager's tests. Glycosides were present in both ethanolic and aqueous extracts. Flavonoids were identified in both ethanolic and aqueous extracts, evidenced by positive results in the lead acetate and alkaline reagent tests. Diterpenes were found only in the ethanolic extract, while phenols were present across all extracts. Proteins were consistently detected in all extracts, and saponins were observed in the ethanolic and aqueous extracts. Carbohydrates were found only in the ethanolic extract. Tannins were present exclusively in the ethanolic extract, and sterols were identified only in

the ethanolic extract. Lignins were absent in all extracts. This screening highlights the diverse range of bioactive compounds in *Sida acuta*, with the ethanolic extract showing the broadest spectrum of constituents, which may contribute to its pharmacological effects.

Table 1, highlight the nephroprotective effects of the ethanolic extract of *Sida acuta* on various biochemical parameters, including serum creatinine, serum urea, blood urea nitrogen (BUN), superoxide dismutase (SOD), and reduced glutathione (GSH). In the control group (Group I), normal levels of serum creatinine (1.533 mg/dL), serum urea (5.4 mg/dL), BUN (12.5 mg/dL), SOD (16.5 U/mg protein), and GSH (5.5 µmol/g tissue) were observed. Conversely, the cisplatin (CP)-induced nephrotoxicity group (Group II) demonstrated a significant elevation in serum creatinine (5.908 mg/dL), serum urea (20.25 mg/dL), and BUN (55.12 mg/dL), indicating severe kidney damage. SOD and GSH levels were markedly reduced to 8.4 U/mg protein and 2.15 µmol/g tissue, respectively, suggesting oxidative stress and impaired antioxidant defense mechanisms.

Treatment with the ethanolic extract of *Sida acuta* significantly attenuated these alterations. In Group III, rats treated with 100 mg/kg of the extract showed a reduction in serum creatinine (3 mg/dL), serum urea (12.1 mg/dL), and BUN (36.41 mg/dL), with an improvement in SOD (12.87 U/mg protein) and GSH (3.99 µmol/g tissue). A higher dose of 200 mg/kg in Group IV further improved these parameters, bringing serum creatinine down to 1.81 mg/dL, serum urea to 9.5 mg/dL, and BUN to 21.58 mg/dL, while SOD and GSH levels rose to 14.7 U/mg protein and 4.58 µmol/g tissue, respectively. These values were comparable to those observed in the gentamycin-treated standard group (Group V), which demonstrated serum creatinine at 1.683 mg/dL, serum urea at 7.8 mg/dL, BUN at 15.6 mg/dL, SOD at 15.23 U/mg protein, and GSH at 5.1 µmol/g tissue.

The results from Table 2 demonstrate the impact of the ethanolic extract of *Sida acuta* on lipid peroxidation, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) levels in cisplatin (CP)-induced nephrotoxicity in rats. In the control group (Group I), normal levels of lipid peroxidation (50.92 nmol/mg), TNF-α (25.5 pg/mL), IL-6 (20.12 pg/mL), and IL-1β (14.5 pg/mL) were observed. Cisplatin treatment (Group II) caused a significant increase in these parameters, with lipid peroxidation rising to 98.23 nmol/mg, TNF-α to 69.53 pg/mL, IL-6 to 75.06 pg/mL, and IL-1β to 85.2 pg/mL. These elevated values indicate oxidative stress, inflammation, and immune response activation due to nephrotoxicity.

Administration of the ethanolic extract of *Sida acuta* significantly mitigated these effects. Rats in Group III, treated with 100 mg/kg of the extract, exhibited reductions in lipid peroxidation (75.46 nmol/mg), TNF-α (41.23 pg/mL), IL-6 (43.2 pg/mL), and IL-1β (38.2 pg/mL), indicating a decrease in oxidative damage and inflammatory markers. A higher dose of the extract (200 mg/kg) in Group IV further improved these parameters, with lipid peroxidation decreasing to 62.27 nmol/mg, TNF-α to 33.68 pg/mL, IL-6 to 36.51 pg/mL, and IL-1β to 28.84 pg/mL. These results were comparable to those in the gentamycin-treated standard group (Group V), which showed lipid peroxidation at 55.34 nmol/mg, TNF-α at 29.5 pg/mL, IL-6 at 25.12 pg/mL, and IL-1β at 18.23 pg/mL.

The significant reduction in lipid peroxidation and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in the treatment groups suggests that the ethanolic extract of *Sida acuta* possesses strong antioxidant and anti-inflammatory properties. These findings support the potential therapeutic role of *Sida acuta* in protecting against CP-induced kidney damage by modulating

oxidative stress and inflammatory pathways.

Table 1: Effect of ethanolic extract of *Sida acuta* on serum creatinine, serum urea, blood urea nitrogen (BUN), super oxide dismutase and reduced glutathione (GSH) parameters

Group	Drug and Dose	Serum creatinine	Serum urea	Blood Urea Nitrogen (BUN)	Super Oxide dismutase	Reduced glutathione (GSH)
		Mean				
Group I	Normal Control (DMSO)	1.533	5.4	12.5	16.5	5.5
Group II	Cisplatin (CP)	5.908	20.25	55.12	8.4	2.15
Group III	CP + Ethanolic extract of *Sida acuta-100*	3	12.1	36.41	12.87	3.99
Group IV	CP + Ethanolic extract of *Sida acuta-200*	1.81	9.5	21.58	14.7	4.58
Group V	CP + Gentamycine (5 mg/kg per day)	1.683	7.8	15.6	15.23	5.1

Table 2: Effect of ethanolic extract of *Sida acuta* on Lipid peroxidation, tumor necrosis factor-alpha (TNF-α), interleukin-6 and interleukin-1β parameters

Group	Drug and Dose	Lipid Peroxidation	Tumor necrosis factor-alpha (TNF-α)	Interleukin-6	Interleukin-1β
		Mean			
Group I	Normal Control (DMSO)	50.92	25.5	20.12	14.5
Group II	Cisplatin (CP)	98.23	69.53	75.06	85.2
Group III	CP + Ethanolic extract of *Sida acuta-100*	75.46	41.23	43.2	38.2
Group IV	CP + Ethanolic extract of *Sida acuta-200*	62.27	33.68	36.51	28.84
Group V	CP + Gentamycine (5 mg/kg per day)	55.34	29.5	25.12	18.23

CONCLUSION

In conclusion, the study demonstrates that the ethanolic extract of *Sida acuta* leaves possesses significant nephroprotective properties against cisplatin-induced nephrotoxicity. Phytochemical analysis revealed a rich profile of bioactive compounds, including alkaloids, flavonoids, and phenols, which are likely contributors to its therapeutic effects. The extract effectively mitigated renal damage, as evidenced by improved levels of serum creatinine, urea, and blood urea nitrogen (BUN). Additionally, it exhibited protective effects against oxidative stress and inflammation by enhancing superoxide dismutase (SOD) and reduced glutathione (GSH) levels, and reducing lipid peroxidation and pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. These results underscore the potential of *Sida acuta* as a valuable therapeutic agent in managing nephrotoxicity and related renal disorders, highlighting its role in traditional medicine and potential for further clinical applications.

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