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PHYTOCHEMICAL SCREENING AND NEPHROPROTECTIVE EFFECTS OF ETHANOLIC EXTRACTS OF *SIDA CORDIFOLIA* LEAVES AGAINST CISPLATIN-INDUCED NEPHROTOXICITY

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ABSTRACT

This study investigated the phytochemical composition and nephroprotective effects of ethanolic extracts of *Sida cordifolia* leaves against cisplatin-induced nephrotoxicity in rats. Phytochemical screening revealed the presence of glycosides, flavonoids, diterpenes, phenols, proteins, saponins, and tannins, particularly in ethanolic and aqueous extracts. The nephroprotective potential was evaluated by measuring serum creatinine, urea, blood urea nitrogen (BUN), oxidative stress markers, and pro-inflammatory cytokines. Cisplatin significantly elevated serum creatinine, urea, BUN, and lipid peroxidation while reducing antioxidant enzymes and increasing inflammation. Treatment with *Sida cordifolia* extract at doses of 100 mg/kg and 200 mg/kg significantly ameliorated these changes in a dose-dependent manner, with the 200 mg/kg dose showing effects comparable to the standard drug, Gentamicin. The extract also restored antioxidant enzyme activity and reduced pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. These findings suggest that *Sida cordifolia* extract has significant nephroprotective potential and could serve as a natural alternative in the prevention of drug-induced nephrotoxicity.

Keywords: *Sida cordifolia*, Nephroprotective, Cisplatin, Oxidative stress, Anti-inflammatory, Flavonoids, Serum creatinine, Lipid peroxidation, Cytokines, Antioxidant enzymes.

INTRODUCTION

Cisplatin, a widely used chemotherapeutic agent, is highly effective in treating various cancers, including ovarian, lung, and bladder cancers. However, its clinical utility is often limited by its dose-dependent nephrotoxicity, which can cause significant kidney damage, leading to increased serum creatinine, urea, and oxidative stress (Yao *et al.,* 2007). Nephrotoxicity induced by cisplatin is associated with the generation of reactive oxygen species (ROS), inflammation, and lipid peroxidation, resulting in renal cell apoptosis and tubular damage (Pabla and Dong, 2008). To mitigate these adverse effects, researchers have increasingly focused on natural plant-based remedies with antioxidant and anti-inflammatory properties.

Sida cordifolia, a medicinal plant widely used in traditional Ayurvedic medicine, has been reported to possess various therapeutic properties, including anti-inflammatory, antioxidant, and nephroprotective effects (Saha *et al.,* 2011). The leaves of *Sida cordifolia* are rich in bioactive compounds such as flavonoids, phenols, and glycosides, which are known to exhibit significant biological activities, including free radical scavenging and inflammation suppression (Sandeep and Nishteswar, 2012). Phytochemical studies of *Sida cordifolia* extracts have revealed the presence of these bioactive constituents, which may play a role in its protective effects against drug-induced toxicity. Given the widespread use of *Sida cordifolia* in traditional medicine and its reported therapeutic potential, this study aimed to evaluate the phytochemical composition and nephroprotective activity of ethanolic extracts of *Sida cordifolia* leaves in a cisplatin-induced nephrotoxicity model in rats. The study hypothesized that the extract would ameliorate kidney damage by reducing oxidative stress, inflammation, and renal dysfunction markers. This research could provide insights into the development of plant-based nephroprotective therapies and contribute to the ongoing search for natural alternatives to mitigate the adverse effects of chemotherapeutic agents.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Sida cordifolia* were collected from Shubham nursery, Bhopal. After collection, plant undergoes washing with tap water to remove the dust, dirt, and other foreign matters attached to the surface of the plant. Wiping the samples with clean and dry cloth enhances the drying process (Handa *et al.,* 2008).

Extraction by soxhlet extraction process

75 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus (Harborne, 1998). The extraction was continued till the defatting of the material had taken place. The air-dried and powdered defatted marc of *Sida cordifolia* were subjected to extraction with chloroform, ethyl acetate, ethanol and water using soxhlet apparatus and allowed to stand for a period of at least 2 days. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods. Phytochemical screening was carried out qualitatively using detection reagents based on the procedures explained in Tiwari *et al.,* 2011, Hanani *et al.,* 2015. The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein.

In-vivo nephrotoxicity Study

Animals

The animal studies were approved by the Institutional Animal Ethics Committee (IAEC),

constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India. In the present study, Wistar rats (150–200 g) were used. During 1 week of acclimatization (22 ± 1°C temperature and 50– 80% humidity), with 12 h cycle variation between the light and dark, freely, animals consumed a standard diet for rodents and water filtered beforehand.

Acute toxicity study

The ethanolic extract of leaves of *Sida cordifolia* was assessed for acute oral toxicity using OECD ANNEX-423 standards. According to prior toxicity studies, ethanolic extract of leaves of *Sida cordifolia* was delivered orally to rats (2000 mg/kg body weight) (Jen *et al.,* 2002).

Experimental design

For the purposes of this intended research, existing studies were slightly modified. There were 7 clusters of 6 rats each, distributed randomly (Alhoshani *et al.,* 2017).

Group I (Control group): acquired daily vehicle (saline, p.o.)

Group II (CP group): provided 4-CP injections (3 mg/kg/day, i.p.) in every five days

Group III (Test group): CP-induced nephrotoxicity rats treated with ethanolic extract of leaves of *Sida cordifolia*-100 mg/kg/p.o. per day

Group IV (Test group): CP-induced nephrotoxicity rats treated with ethanolic extract of leaves of *Sida cordifolia*-200 mg/kg/p.o. per day

Group VII (STD group): CP-induced nephrotoxicity rats were treated with Gentamycin (5 mg/kg per day)

Biochemical assessment was carried out by sacrificing the animals at the last day of study.

Biochemical Assessment

A blood sample using the retro-orbital plexus was collected and centrifuged for 20 minutes at 1000 rpm in direction to dispersed the serum. Last day of the experiment, urine samples were collected from 24-hour urine samples. Biochemical analysis was then conducted on both samples carried out by standard methods (Kpemissi *et al.,* 2019).

RESULTS AND DISCUSSION

The phytochemical analysis of the different solvent extracts of *Sida cordifolia* leaves revealed the presence of important bioactive compounds that could contribute to its medicinal properties. As seen in Table 1, the ethanolic extract showed the presence of glycosides, flavonoids, diterpenes, phenols, proteins, carbohydrates, saponins, and tannins. The aqueous extract shared similar phytochemical constituents, indicating that water-based extraction also yields significant bioactive compounds. In contrast, the chloroform and ethyl acetate extracts demonstrated fewer phytochemicals, primarily showing proteins and flavonoids. This suggests that ethanol and water are more effective solvents for extracting bioactive components from *Sida cordifolia*.

The presence of flavonoids and phenols, known for their antioxidant properties, supports the use of *Sida cordifolia* in traditional medicine for conditions associated with oxidative stress. The detection of glycosides and saponins, both known for their pharmacological effects, further

indicates that *Sida cordifolia* may possess therapeutic potential for a range of ailments, particularly those involving the kidneys and oxidative damage.

The ethanolic extract of *Sida cordifolia* was evaluated for its nephroprotective effects in rats subjected to cisplatin-induced nephrotoxicity, with various renal function parameters analyzed. Cisplatin, a chemotherapeutic agent, is known to induce nephrotoxicity, as evidenced by elevated levels of serum creatinine, urea, and blood urea nitrogen (BUN), alongside increased oxidative stress and inflammation.

In Table 2, cisplatin administration caused a significant increase in serum creatinine (5.908±0.8667 mg/dl), a key marker of kidney damage, compared to the normal control (1.533±0.1418 mg/dl). Treatment with the ethanolic extract of *Sida cordifolia* at 100 mg/kg and 200 mg/kg reduced the elevated creatinine levels to 3.2±0.56 mg/dl and 1.8±0.4372 mg/dl, respectively, indicating a dose-dependent nephroprotective effect. The higher dose produced a creatinine level comparable to the gentamicin-treated group (1.683±0.09766 mg/dl), a standard nephroprotective drug, suggesting that *Sida cordifolia* has substantial potential as a natural nephroprotective agent.

Similarly, cisplatin caused a marked increase in serum urea levels (20.25±0.5 mg/dl) as shown in Table 3, indicating impaired kidney function. Treatment with the ethanolic extract significantly reduced serum urea, with the 200 mg/kg dose lowering urea to 9.8±0.28 mg/dl, approaching the levels seen in the gentamicin-treated group (7.8±0.22 mg/dl). The reduction in urea levels further confirms the protective role of *Sida cordifolia* in mitigating cisplatin-induced renal damage.

The effect of *Sida cordifolia* on BUN, another indicator of kidney function, is summarized in Table 4. Cisplatin treatment raised BUN levels to 55.12±3.27 mg/dl, while the extract at 100 mg/kg and 200 mg/kg significantly reduced BUN levels to 35.21±2.6 mg/dl and 20.12±1.91 mg/dl, respectively. The higher dose again showed results similar to gentamicin, suggesting that *Sida cordifolia* is effective in preventing nitrogenous waste buildup in the blood.

Oxidative stress plays a key role in the pathogenesis of nephrotoxicity. In cisplatin-treated rats, the levels of superoxide dismutase (SOD) and reduced glutathione (GSH), both critical antioxidants, were significantly decreased (8.4±0.18 U/mg and 2.15±0.15 U/mg, respectively). However, the ethanolic extract of *Sida cordifolia* increased SOD levels to 14.5±0.33 U/mg at 200 mg/kg, as seen in Table 5, and improved GSH levels to 4.56±0.24 U/mg (Table 6). These results indicate the extract's ability to restore antioxidant defenses, which is crucial in protecting kidney tissues from oxidative damage.

Additionally, lipid peroxidation, a marker of oxidative damage to cell membranes, was significantly elevated by cisplatin (98.23±0.9 nmol/mg). Treatment with the extract reduced lipid peroxidation levels to 61.23±0.57 nmol/mg at the 200 mg/kg dose (Table 7), demonstrating its efficacy in minimizing membrane damage and preserving cell integrity.

Inflammation is another key factor in cisplatin-induced nephrotoxicity, as seen in the elevated levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Cisplatin increased TNF-α to 69.53±1.5 pg/ml (Table 8), while treatment with *Sida cordifolia* extract at 200 mg/kg significantly reduced TNF-α to 32.8±1.35 pg/ml, approaching the levels seen in gentamicin-

treated rats (29.5±1.31 pg/ml). Similar reductions were observed for IL-6 (75.06±4.8 pg/ml to 35.21±2.7 pg/ml, Table 9) and IL-1β (85.2±3.8 pg/ml to 28.54±2.1 pg/ml, Table 10). These reductions in pro-inflammatory cytokines suggest that *Sida cordifolia* exerts significant anti-inflammatory effects, further supporting its nephroprotective properties.

Table 1: Result of phytochemical screening of leaves extract of *Sida cordifolia*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**				
	Wagner's Test:	-ve	-ve	-ve	-ve
	Hager's Test:	-ve	-ve	+ve	-ve
2.	**Glycosides**				
	H_2SO_4 Test:	-ve	-ve	+ve	-ve
3.	**Flavonoids**				
	Lead acetate Test:	-ve	-ve	+ve	+ve
	Alkaline reagent test:	-ve	+ve	+ve	+ve
4.	**Diterpenes**				
	Copper acetate Test:	-ve	-ve	+ve	+ve
5.	**Phenol**				
	Ferric Chloride Test:	-ve	+ve	+ve	+ve
	Folin Ciocalteu Test:	-ve	-ve	-ve	-ve
6.	**Proteins**				
	Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	**Carbohydrate**				
	Fehling's Test:	-ve	-ve	-ve	+ve
	Benedicts Test:	-ve	-ve	+ve	-ve
8.	**Saponins**				
	Froth Test:	-ve	-ve	+ve	+ve
9.	**Tannins**				
	Gelatin test:	-ve	-ve	+ve	-ve
10.	**Sterols**				
	Salkowski's Test:	-ve	-ve	-ve	-ve
11.	**Lignins**				
	Labat test:	-ve	-ve	-ve	-ve

Table 2: Effect of Ethanolic extract of leaves of *Sida cordifolia* on serum creatinine

Group	Drug and Dose	Serum creatinine	
		Mean	SEM
Group I	Normal Control (DMSO)	1.533	0.1418
Group II	Cisplatin (CP)	5.908	0.8667
Group III	CP + *Ethanolic extract of leaves of Sida*	3.2	0.56

	cordifolia-100		
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	1.8	0.4372
Group V	CP + Gentamycine (5 mg/kg per day)	1.683	0.09766

Table 3: Effect of Ethanolic extract of leaves of *Sida cordifolia* on serum urea

Group	Drug and Dose	Serum urea	
		Mean	SEM
Group I	Normal Control (DMSO)	5.4	0.21
Group II	cisplatin (CP)	20.25	0.5
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	11.5	0.35
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	9.8	0.28
Group V	CP + Gentamycine (5 mg/kg per day)	7.8	0.22

Table 4: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Blood Urea Nitrogen (BUN)

Group	Drug and Dose	Blood Urea Nitrogen (BUN)	
		Mean	SEM
Group I	Normal Control (DMSO)	12.5	0.65
Group II	cisplatin (CP)	55.12	3.27
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	35.21	2.6
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	20.12	1.91
Group V	CP + Gentamycine (5 mg/kg per day)	15.6	0.77

Table 5: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Super Oxide dismutase

Group	Drug and Dose	Super Oxide dismutase	
		Mean	SEM
Group I	Normal Control (DMSO)	16.5	0.29
Group II	cisplatin (CP)	8.4	0.18

Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	12.63	0.27
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	14.5	0.33
Group V	CP + Gentamycine (5 mg/kg per day)	15.23	0.35

Table 6: Effect of Ethanolic extract of leaves of *Sida cordifolia* on reduced glutathione (GSH)

Group	Drug and Dose	Reduced glutathione (GSH)	
		Mean	SEM
Group I	Normal Control (DMSO)	5.5	0.27
Group II	cisplatin (CP)	2.15	0.15
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	3.95	0.22
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	4.56	0.24
Group V	CP + Gentamycine (5 mg/kg per day)	5.1	0.25

Table 7: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Lipid Peroxidation

Group	Drug and Dose	Lipid Peroxidation	
		Mean	SEM
Group I	Normal Control (DMSO)	50.92	0.61
Group II	cisplatin (CP)	98.23	0.9
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	73.26	0.67
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	61.23	0.57
Group V	CP + Gentamycine (5 mg/kg per day)	55.34	0.45

Table 8: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Tumor necrosis factor-alpha (TNF-α)

Group	Drug and Dose	Tumor necrosis factor-alpha (TNF-α)	
		Mean	SEM
Group I	Normal Control (DMSO)	25.5	1.31

Group II	cisplatin (CP)	69.53	1.5
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	39.65	1.4
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	32.8	1.35
Group V	CP + Gentamycine (5 mg/kg per day)	29.5	1.31

Table 9: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Interleukin-6

Group	Drug and Dose	Interleukin-6	
		Mean	SEM
Group I	Normal Control (DMSO)	20.12	1.2
Group II	cisplatin (CP)	75.06	4.8
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	41.2	2.9
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	35.21	2.7
Group V	CP + Gentamycine (5 mg/kg per day)	25.12	1.5

Table 10: Effect of Ethanolic extract of leaves of *Sida cordifolia* on Interleukin-1β

Group	Drug and Dose	Interleukin-1β	
		Mean	SEM
Group I	Normal Control (DMSO)	14.5	1.2
Group II	cisplatin (CP)	85.2	3.8
Group III	CP + *Ethanolic extract of leaves of Sida cordifolia-100*	37.82	2.4
Group IV	CP + *Ethanolic extract of leaves of Sida cordifolia-200*	28.54	2.1
Group V	CP + Gentamycine (5 mg/kg per day)	18.23	1.5

CONCLUSION

The results of this study demonstrate that the ethanolic extract of *Sida cordifolia* possesses significant nephroprotective effects against cisplatin-induced kidney damage. The extract effectively reduced serum creatinine, urea, BUN, and oxidative stress markers while also attenuating the inflammatory response. These findings suggest that *Sida cordifolia* could be a promising natural therapeutic agent for the prevention and treatment of nephrotoxicity, with effects comparable to standard treatments like gentamicin. Further studies to isolate and characterize the active compounds responsible for these effects may provide valuable insights into the development of plant-based nephroprotective therapies.

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