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Research Article

Nephroprotective Effect of *Spermacoce hispida* by reducing Oxidative and Nitrosative Stress in Experimental Model

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Abstract

Present study was aimed to investigate the protective of *Spermacoce hispida* in Cisplatin induced nephrotoxicity using *in vivo* model. *S. hispida* was collected and extract of plant was prepared in different solvents and prepared extracts was used for phytochemical screening. Nephrotoxicity in rats was induced by a single intraperitoneal injection of Cisplatin at a dose of 5 mg/kg. *S. hispida* extract in different solvent at the dose 100 mg/kg used to find protective activity. Blood urea nitrogen (BUN), serum creatinine, oxidative stress, a proinflammatory cytokine, NO, and histological alteration was measured to estimate the therapeutic ability of *S. hispida*. BUN, creatinine, and inflammatory cytokine levels in rats were increased by Cisplatin but decreased by *S. hispida* treatment for 14 days. Besides, *S. hispida* treatment decreased oxidative stress and nitric oxide in Cisplatin-treated rats. Cisplatin treated rats shows altered structure of kidney tissue; meanwhile *S. hispida* normalizes the structure of kidney tissue. From this study, we conclude that *S. hispida* has nephroprotective activity by inhibiting the oxidative stress and NO production in rats from nephrotoxicity.

Keywords: *Spermacoce hispida*; Cisplatin; oxidative stress; Cytokine; Nitric oxide.

INTRODUCTION

Cisplatin (CP) is a chemotherapeutic that is extensively used to treat cancers such as heads, neck, vaginal, and ovarian tumours, although it is linked to nephrotoxicity in 28–36 percent of individuals who get an initial dose of Cisplatin (50–100 mg/m²).^{1,2} Nephrotoxicity is produced by the buildup of excessive levels of Cisplatin in the kidneys. Because of this significant consequence, its clinical use has been restricted. In the case of worsening renal failure, cisplatin intermission remains the only option.^{3,4} Cisplatin-induced nephrotoxicity is caused by necrosis and apoptosis, as well as endothelial factors and tubular inflammation. Because of the oxidative stress caused by Cisplatin, renal tubule damage develops.⁵ The morphology and physiology of cellular membranes are altered by the generation of reactive oxygen and nitrogen species (ROS and RNS).⁶ The mechanisms for CP-induced acute nephropathy are explained by their accumulation in the kidney and cell organelles. Although many pathways for CP-induced renal injury have been explored, including mitochondrial malfunction, inflammation, DNA damage, oxidative stress, and cell death, the precise mechanism remains unknown.⁷ The healing of injured tissues is closely linked to several cytokines. Interferons, interleukins (IL-6, IL-10, IL-1), tumour necrosis factor (TNF-), and various growth factors have all demonstrated their ability to serve as

nephrotoxicity biomarkers because they are involved in glomerular and tubular damage and restoration.⁸ As a result, free radical scavengers and antioxidants can help to avoid nephrotoxicity caused by cisplatin. *Spermacoce hispida* (Rubiaceae) was called in Tamil as "Nattaiccuri" and in English as "Shaggy button weed." The plant's seed extract has long been utilised as a cure for a variety of diseases, including nerve and kidney problems.⁹ Antioxidant, anti-inflammatory, analgesic, hypolipidemic, antihypertensive, and other pharmacological actions have been reported for this plant.^{10,11} From this background present study was aimed to find the protective effect of *Spermacoce hispida* in Cisplatin-induced nephrotoxicity in rats by estimating oxidative and nitrosative stress, cytokine, kidney injury markers, and histological examination.

MATERIAL AND METHODS

Collection and Authentication of Plant:

The plant *S. hispida* was collected from the fields in Buldhana, District, Maharashtra, India. The plant material was authenticated by Dr. Vanita U. Pochhi, Shri Shivaji Science and Arts College Chikhali, Dist- Buldhana, Maharashtra, India.

Preparation of extracts:

Fresh *S. hispidula* plants were harvested, cut into small pieces, and dried for fourty days in the shade at room temperature. The powdered plant parts were sieved after being pounded to a fine powder. Fine powder was used for the preparation of extracts of different solvents. The powdered sample was subjected to successive solvent extraction taking from hexane, chloroform, ethyl acetate, methanol, and water. 50gm of the sample was subjected to Soxhlet extraction for 8 hrs with 500ml of the various solvents. Then, the excessive solvents were removed by using Rotary vacuum evaporator (MAC Buchi type). These extracts were stored in desiccators for further analysis.¹¹

$$\% \text{ yeild} = \frac{\text{Weight of residue obtained}}{\text{weight of plant material taken}}$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.¹²

Pharmacological screening

Animals

The study was pre-approved by the Institutional Animal Ethics Committee (IAEC) of the College. Protocol approval No. 751/PO/Re/S/03/CPCSEA. Male adult Wistar rats weighing approximately a 180- 220 gm were acquired from vital animal residence facility and maintained with fashionable conditions of humidity (60-65%), temperature (20 ± 2 °C), light and dark cycle 12 hr. Food and water have been freely accessible to animals.

Chemicals and Drugs

Cisplatin was obtained from a local pharmacy vendor. Creatinine kit, BUN kit, and total protein kit were received from ERBA, India. Cytokine kits were obtained from Loba Chemicals Pvt Ltd. Sodium chloride, dibasic potassium phosphate; monobasic potassium phosphate, HCL, phosphoric acid, and Sterile saline were used in the current study.

Experimental design

Animals were randomly divided into five groups each containing six.

Group I: Normal - Received vehicle

Group II: Control- Received Cisplatin 5 mg/kg i.p single dose on the 10th day of study.

Group III to VII- Received *Spermacoce hispidula* extract of hexane, chloroform, aqueous, ethyl acetate, and methanol 100 mg/kg respectively for 14 days

Nephrotoxicity was induced in all animals except normal group by single intraperitoneal injection of Cisplatin on the 10th day of study.

Animals were weighted and given ether anaesthesia after 24 hours of their last treatment. A blood sample was taken from each animal, and serum was isolated. Each animal's kidney was removed and cleaned with ice cold saline. For homogenate preparation and histological investigation, the kidneys were chopped into small pieces.¹³

Measurement of body weight

Before starting the experiment, the body weight of each animal was measured, as well as after 24 hours of the final treatment.¹⁴

Determination of blood urea nitrogen (BUN) and serum creatinine

The concentrations of blood urea nitrogen and serum creatinine were measured according to the manufacturer's instructions. BUN absorbance was measured at 620 nm, and the final BUN and serum creatinine concentrations were estimated using the standard curve.¹⁵

Estimation of Oxidative Stress

Estimation of Malondialdehyde of lipid peroxidation in kidney tissue

The most crucial mode from membrane lipid peroxidation as malondialdehyde (MDA) concentration in kidney tissues was measured as previously described method. The precept of lipid peroxidation depends on the pink color formation due to reaction among MDA and thiobarbituric acid. Absorbance of pink color become measured spectrophotometrically at 532 nm.¹⁶

Estimation of Reduced Glutathione (GSH)

Glutathione concentration in kidney tissues homogenate was measured as previously described technique by Jain et al., 2020.¹⁷

Estimation of Superoxide Dismutase (SOD) Activity

The kidney homogenate (10 µl) was added in the combination of 20 µl of 500 mM/1 of sodium carbonate, 1 ml of 0.3% Triton X-100, 10 µL of 1.0 mM/1 of EDTA, 2.5 ml of 10 mM/1 of hydroxylamine, and 89 ml of distilled water. To this reaction aggregate, 10 µl of 240 µM/1 of NBT was added and subsequently optical density of this reaction mixture was measured at 560 nm in kinetic mode.¹⁸

Determination of cytokine level

Using ELISA kits, the concentration of cytokine-like IL-6, IL-1 beta, and TNF- in kidney tissue homogenate were determined according to the manufacturer's procedure. A standard curve was used to determine the final concentration.¹⁹

Estimation of Nitric Oxide

NO concentrations were measured using blood serum samples. For estimation of NO, 50 µl of serum sample mixed with 50 µl of Griess reagent and the absorbance was taken at 540 nm using a spectrophotometer. Sodium nitrite was used to prepare a standard calibration curve. The concentration of NO becomes expressed as µM/mg.²

Histopathology

The kidney tissues were fixed in formalin (10%) solution and embedded in paraffin. Serial thin sections (4 µm) had been taken using a microtome. The sections have been stained with hematoxylin and eosin (H&E). Sections have been examined under the microscope and finally photos have been taken.²⁰

Statistical analysis

Data were expressed as Mean ± SEM, data were analyzed by one way ANOVA, followed by Bonferroni's post hoc test for comparison through a graph pad, prism software, and version 6.0, USA. The value of P < 0.05 was considered significant.

RESULTS

Percentage yield of different solvent extracts of *S. hispidula*

The plant powder of 1 g was subjected to extract the active phytochemicals with five different solvents, such as hexane, chloroform, ethyl acetate, methanol and aqueous using Soxhlet apparatus. The solvent was removed by rotary evaporator under reduced pressure at 40 oC, which yielded thick colloidal

extracts. *Spermacoce hispida* (100 g) and yield of the bioactive principle was maximum in methanol extract (6.32%), followed by ethyl acetate (4.58%) and chloroform (4.63%). The yield was only 1.19% and 2.05% in aqueous and hexane extract respectively. The color of extracts ranged from light green to light brown, the consistency was between powder and that of a paste.

Screening of phytochemicals from *S. hispida*

The preliminary screening of phytochemicals from *S. hispida* revealed that, presence of various active components such as alkaloids, carbohydrates, flavonoid, glycosides, phenols,

quinones, saponins, steroids, tannins, terpenoids and triterpenoids to a greater extent in the polar solvents.

Effect of *S. hispida* on the body weight

At the end of the study, Cisplatin treated group (179.3 ± 6.103) confirmed significantly decreased body weight compared to the normal group (192.9 ± 4.595) ($P < 0.05$). Meanwhile, the administration of *S. hispida* with extract of different solvent found an increase in the body weight in comparison with Cisplatin group. Administration of *S. hispida* (100 mg/kg of methanol extract) shows maximum increase in body weight (198.0 ± 4.264) as compared to Cisplatin group ($p < 0.05$) given in table 1.

Table 1: Effect of *S. hispida* extracts on change in body weight in Cisplatin-induced Nephrotoxicity rats

Group	Body weight		Relative kidney weight
	Initial on 0 day	Last on 14 th day	
Normal	187.5 ± 3.526	192.9 ± 4.595	1.929 ± 0.025
Cisplatin	188.6 ± 3.347	179.3 ± 6.103	3.641 ± 0.176
SH Hexane	190.2 ± 3.095	193.1 ± 3.362	2.701 ± 0.315
SH Chloroform	205.2 ± 3.174	206.1 ± 5.423	2.418 ± 0.037
SH Aqueous	205.4 ± 4.920	207.4 ± 5.390	2.956 ± 0.084
SH Ethyl acetate	191.2 ± 4.362	194.4 ± 3.069	2.515 ± 0.278
SH Methanol	189.3 ± 3.024	198.0 ± 4.264	2.340 ± 0.082

Effect of *S. hispida* on the blood urea nitrogen (BUN) and serum creatinine

Cisplatin treated rats shows significant increased the level of BUN (121.4 ± 1.317 mg/dl) and serum creatinine (6.675 ± 0.1750 mg/dl) compared to normal rats (42.28 ± 1.327 mg/dl and 1.400 ± 0.1080 mg/dl respectively ($P < 0.001$). *S. hispida*

with extract in different solvent treated rats showed significant changes in biochemical markers compared to Cisplatin group. However, the administration *S. hispida* with methanol and ethyl acetate extract shows maximum decrease in level as compared to other solvent extract shown in figure 1.

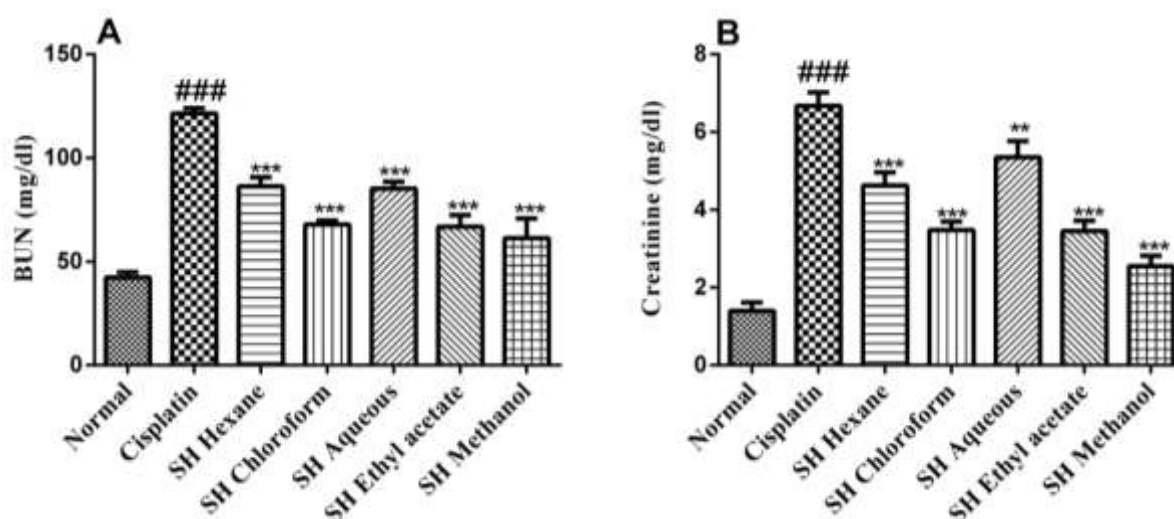


Figure 1: Effect of *S. hispida* extracts on A) BUN, B) Creatinine in Cisplatin-induced Nephrotoxicity rats

Effect of *S. hispida* on the Oxidative stress

Cisplatin treated rat's shows a significant elevation in MDA level and reduction in GSH content ($P < 0.05$). Cisplatin treated rats showed a notably lowered level of SOD as compared to normal rats ($P < 0.05$). Treatment with *S. hispida* extract

protected the rat kidney tissue from the lipid peroxidation caused by cisplatin. In the case of GSH, there has been a tremendous increase in GSH levels and the activities of SOD in the rats receiving *S. hispida* extract in different solvent shown in figure 2.

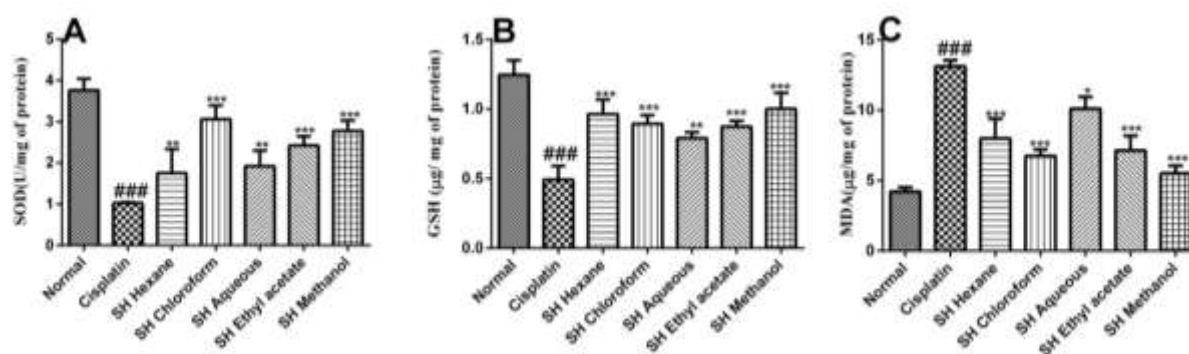


Figure 2: Effect of *S. hispida* extracts on A) SOD, B) GSH, C) MDA in Cisplatin-induced Nephrotoxicity rats

Effect of *S. hispida* on the cytokine release

We determined the level of pro-inflammatory cytokines like TNF- α , IL-6, and IL-1 β in the kidney tissue homogenates. Rats treated with Cisplatin significantly ($P < 0.05$) increased the level of cytokines as TNF- α , IL-6, and IL-1 β in the kidney tissue homogenate in comparison to the normal rats ($P <$

0.05). Treatment with *S. hispida* in different solvent shows reduced the level of cytokine compared with the Cisplatin group. The 100 mg/kg of *S. hispida* methanol extract treated group showed a more prominent effect compared with the rats treated with rats treated with other extract shown in figure 3.

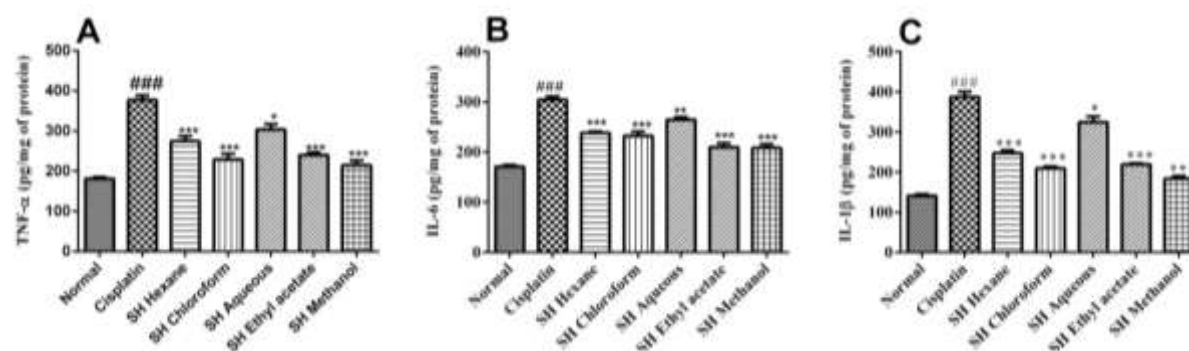


Figure 3: Effect of *S. hispida* extracts on A) TNF-alpha, B) IL-6, C) IL-1beta in Cisplatin-induced Nephrotoxicity rats

Effect of *S. hispida* on the Nitric oxide

Cisplatin treatment significantly increased the level of nitric oxide ($505.2 \pm 7.23 \mu\text{M/mg}$) as compared to normal groups ($235.5 \pm 4.455 \mu\text{M/mg}$) and Groups treated with *S. hispida*

extract in different solvent at dose 100 mg/kg showed a decrease in nitric oxide level. Methanolic extract of *S. hispida* shows more potent activity than other solvent extract shown in figure 4.

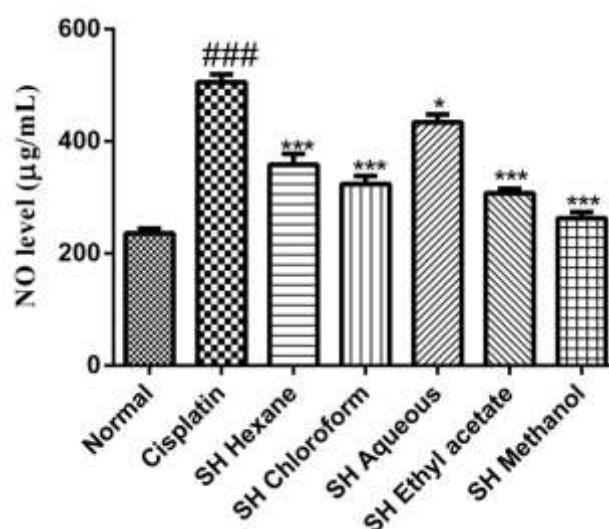


Figure 4: Effect of *S. hispida* extracts on nitric oxide in Cisplatin-induced Nephrotoxicity rats

Effect of *S. hispida* on Histopathology

The microscopic exam of renal tissues in the normal group found regular glomerulus shape and renal tubular interstitial with no evidence of cellular necrosis and inflammatory infiltration. The Rats injected with cisplatin confirmed with

necrosis and dropping of renal tubular epithelial cells, vacuolization of the renal cortex, and inflammatory infiltrations. In contrast, *S. hispida* treatment reduced the wide variety of cell infiltrate. The renal tubular necrosis score was protected by using treatment of *S. hispida* in different solvents compared to the cisplatin group shown in figure 5.

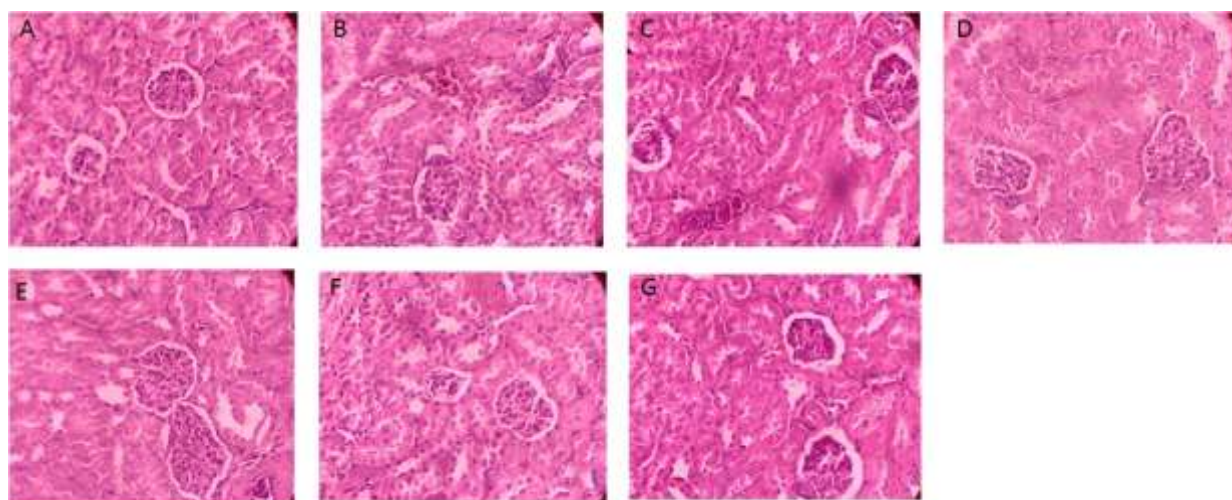


Figure 5: Effect of *S. hispida* extracts on histopathology of kidney in Cisplatin-induced Nephrotoxicity rats

A) Normal, B) Cisplatin, C) SH Hexane, D) SH Chloroform, E) SH Aqueous, F) Ethyl acetate, G) SH Methanol

DISCUSSION:

Cisplatin is widely used as an antineoplastic agent but its therapeutic potential is limited because of its nephrotoxicity effect. A single dose of Cisplatin in cancer treatment shows approximately 40% of patients suffering from renal dysfunction caused due to Cisplatin.²¹ The therapeutic effect of *S. hispida* in Cisplatin-induced on nephrotoxicity and its molecular mechanism has no longer been proved yet.

S. hispida was collected and extract were prepared in different solvent like chloroform, hexane, methanol, aqueous, ethyl acetate, among this extract methanolic extract was found with maximum percentage yield. In case of phytochemical screening, alkaloids, flavonoids, carbohydrates, proteins, phenols, steroid, glycosides were found in plant.

In the present study, we investigated the potential protective effect of *S. hispida* against cisplatin-induced nephrotoxicity through experimental models.

BUN and serum creatinine are kidney damage markers and hence serve as cell injury diagnostic markers. When the cell membrane becomes more permeable or ruptures, these indicators are likely to be discharged into the bloodstream from injured tissue. The most common source of high levels of these renal injury indicators is damaged renal tissue.²² Cisplatin injected rats showed an increased level of BUN and serum creatinine. Treatment with *S. hispida* inhibited the increase in the level of renal injury markers. Several studies have found that oxidative damage generated by ROS plays a role in the etiology of nephrotoxicity.¹⁴ In this study, we recognized the importance of oxidative stress in the improvement of cisplatin-induced nephrotoxicity via *S. hispida*. Those phenomena were effectively reversed with the *S. hispida* treatment for fourteen days. Similarly to oxidative stress, inflammation performs an essential component within the pathogenesis of cisplatin-prompted nephrotoxicity.²³

TNF- is a classic inflammatory mediator that stimulates neutrophils and macrophages, resulting in cytokine

production and irritation, ultimately leading to cell necrosis. Furthermore, IL-1 induces inflammation, causes fever, and encourages immune system development and differentiation.²⁴ The inhibitory effect exhibited by *S. hispida* may contribute to the suppression of the inflammation associated with excessive secretion of TNF-alpha, IL-6, and IL-1β induced by Cisplatin due to loss of renal function.

In this work, we found that Cisplatin-injected rats had a higher level of nitric oxide than previously reported in many publications.²⁵ Treatment with *S. hispida*, on the other hand, reversed the level of nitric oxide production. Degenerative changes within the proximal tubules, such as hydropic degeneration, extended cytoplasmic vesicles, cytoplasmic vacuolization, pycnotic nuclei, obvious loss of the brush border, necrosis and apoptosis of tubular cells, and desquamation of necrotic epithelial cells filling the tubules in renal tissue caused by Cisplatin.²⁶ The same effect was observed in Cisplatin injected rats. *S. hispida* treated rats prevented the damage of renal tissue.

CONCLUSION:

From this study we conclude that, *S. hispida* contains different phytochemical like alkaloids, glycosides, carbohydrates, flavonoids, steroids, proteins etc. Prepared extract in different solvent shows protective effect in Cisplatin induced Nephrotoxicity by maintaining oxidative and nitrostatic stress.

Conflict of Interest: Author has no conflict of interest

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