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Protective Effects of Spermacoce hispida Against Cisplatin-Induced Nephrotoxicity: A Study on Oxidative and Nitrosative Stress

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Abstract: *Background: Nephrotoxicity is a common and severe side effect of cisplatin, a widely used chemotherapeutic agent. The mechanism of cisplatin-induced nephrotoxicity involves oxidative stress, inflammation, and apoptosis, leading to renal damage. There is growing interest in exploring natural products with antioxidant and anti-inflammatory properties as potential protective agents against drug-induced nephrotoxicity.Objective: The present study aimed to investigate the protective effects of Spermacoce hispida against cisplatin-induced nephrotoxicity in an in vivo rat model.*
Methods: S. hispida was collected, and plant extracts were prepared using different solvents. The prepared extracts underwent phytochemical screening. Nephrotoxicity was induced in rats through a single intraperitoneal injection of cisplatin at a dose of 5 mg/kg. S. hispida extracts at a dose of 100 mg/kg were administered to assess their protective activity. Key parameters measured included blood urea nitrogen (BUN), serum creatinine, oxidative stress markers, proinflammatory cytokines, nitric oxide (NO) levels, and histological alterations in kidney tissue.Results: Cisplatin treatment resulted in increased levels of BUN, serum creatinine, and proinflammatory cytokines in rats, indicating nephrotoxicity. However, treatment with S. hispida extracts for 14 days significantly decreased these elevated levels. Additionally, S. hispida treatment reduced oxidative stress and NO production in cisplatin-treated rats. Histological examination revealed that cisplatin induced structural damage in kidney tissues, which was normalized by S. hispida treatment.
Conclusion: The study concludes that Spermacoce hispida exhibits nephroprotective activity, likely by inhibiting oxidative stress and NO production, thereby mitigating cisplatin-induced nephrotoxicity in rats.

Keywords: Cisplatin; oxidative stress;. Spermacoce hispida; Cytokine; Nitric oxide; HEK-293 cell line

I. INTRODUCTION

Cisplatin, a platinum-based chemotherapeutic agent, is widely used in the treatment of various malignancies, including testicular, ovarian, bladder, and lung cancers. Despite its clinical efficacy, cisplatin is notorious for its dose-limiting nephrotoxicity, which manifests as acute kidney injury (AKI) in patients. The primary mechanisms underlying cisplatin-induced nephrotoxicity include oxidative stress, inflammation, and apoptosis, leading to significant renal damage. This nephrotoxicity not only limits the therapeutic potential of cisplatin but also severely impacts patients' quality of life, necessitating the exploration of effective nephroprotective strategies.

In recent years, there has been a growing interest in natural products as potential therapeutic agents due to their diverse pharmacological properties and lower toxicity profiles compared to synthetic drugs. Among these, medicinal plants with known antioxidant and anti-inflammatory properties are being increasingly investigated for their protective effects against drug-induced nephrotoxicity. *Spermacoce hispida* (also known as *Borreria hispida*), a medicinal plant traditionally used in various cultures for its therapeutic benefits, has shown promise due to its rich phytochemical content, including flavonoids, phenolic compounds, and alkaloids. These compounds are known for their potent antioxidant and anti-inflammatory effects, making *S. hispida* a candidate for nephroprotection.

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Research Gap:

Despite the well-documented pharmacological properties of *Spermacoce hispida*, its potential role in protecting against cisplatin-induced nephrotoxicity has not been extensively studied. Most of the existing research on *S. hispida* has focused on its general antioxidant, anti-inflammatory, and antimicrobial activities, with limited exploration into its specific application in renal protection. Additionally, while several natural products have been investigated for their nephroprotective effects, there remains a need for more comprehensive studies that not only assess the biochemical parameters but also include detailed histopathological evaluations to confirm tissue-level protection.

Novelty:

The present study is novel in its approach to investigating the nephroprotective effects of *Spermacoce hispida* against cisplatin-induced nephrotoxicity using an in vivo rat model. The study is distinctive in several aspects:

- **Comprehensive Evaluation:** Unlike previous studies, this research integrates both biochemical and histopathological analyses to provide a holistic understanding of the protective effects of *S. hispida* on kidney function and structure.
- **Dose and Duration:** The study explores the effects of *S. hispida* extracts at a specific dose of 100 mg/kg over a 14-day treatment period, providing insights into the optimal dosage and duration for therapeutic efficacy.
- **Mechanistic Insights:** By focusing on oxidative stress markers, proinflammatory cytokines, and nitric oxide (NO) production, the study delves into the underlying mechanisms by which *S. hispida* exerts its nephroprotective effects, thereby offering potential pathways for further research and therapeutic development.
- **Histological Confirmation:** The inclusion of detailed histological analysis to observe structural alterations in kidney tissues provides concrete evidence of the protective effects of *S. hispida*, bridging the gap between biochemical changes and actual tissue protection.

In summary, this study not only fills a significant research gap by exploring the nephroprotective potential of *Spermacoce hispida* in the context of cisplatin-induced nephrotoxicity but also offers novel insights into the mechanisms underlying its protective effects, paving the way for future research and clinical applications.

II. MATERIAL AND METHODS

Collection and Authentication of Plant:

Spermacoce hispida plants were collected from their natural habitat and subsequently authenticated by a qualified institution to ensure proper identification and validity of the plant material.

Preparation of extracts:

After collecting the fresh S. hispida plants, they were sliced into tiny pieces and left to dry at room temperature in the shade for forty days. Following their reduction to a fine powder, the plant components were passed through a screen. To make extracts from various solvents, fine powder was used. A series of solvent extractions using hexane, chloroform, ethyl acetate, methanol, and water were performed on the powdered material. Fifty grams of the material was Soxhlet extracted with 500 milliliters of each solvent for eight hours. After that, a Rotary vacuum evaporator of the MAC Buchi type was used to extract the surplus solvents. Desiccators were used to keep these extracts for future research.[11].

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

Phytochemical Screening

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

Pharmacological screening

Animals

The study received approval from the Institutional Animal Ethics Committee (IAEC). Male adult Wistar rats, weighing between 180 and 220 grams, were sourced from a certified animal facility and were kept under controlled conditions with a humidity range of 60-65%, a temperature of 20 ± 2°C, and a 12-hour light/dark cycle. The animals had unrestricted access to food and water.

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Chemicals and Drugs

A local drug seller provided the cisplatin. Sigma Aldrich was the source of the formononetin. We obtained the creatinine, BUN, and total protein kits from ERBA in India. Loba Chemicals Pvt Ltd. supplied the cytokine kits. The present investigation made use of the following chemicals: sterile saline, sodium chloride, monobasic potassium phosphate, HCL, phosphoric acid, and dibasic potassium phosphate.

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 10^{th} day of study.

Group III to VII- Received *Spermacoce hispida* 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 10^{th} 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[20].

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Histopathology

The kidney tissues were fixed in formalin (10%) solution and embedded in paraffin. Serial skinny 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.

III. RESULTS

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

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 14th 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.

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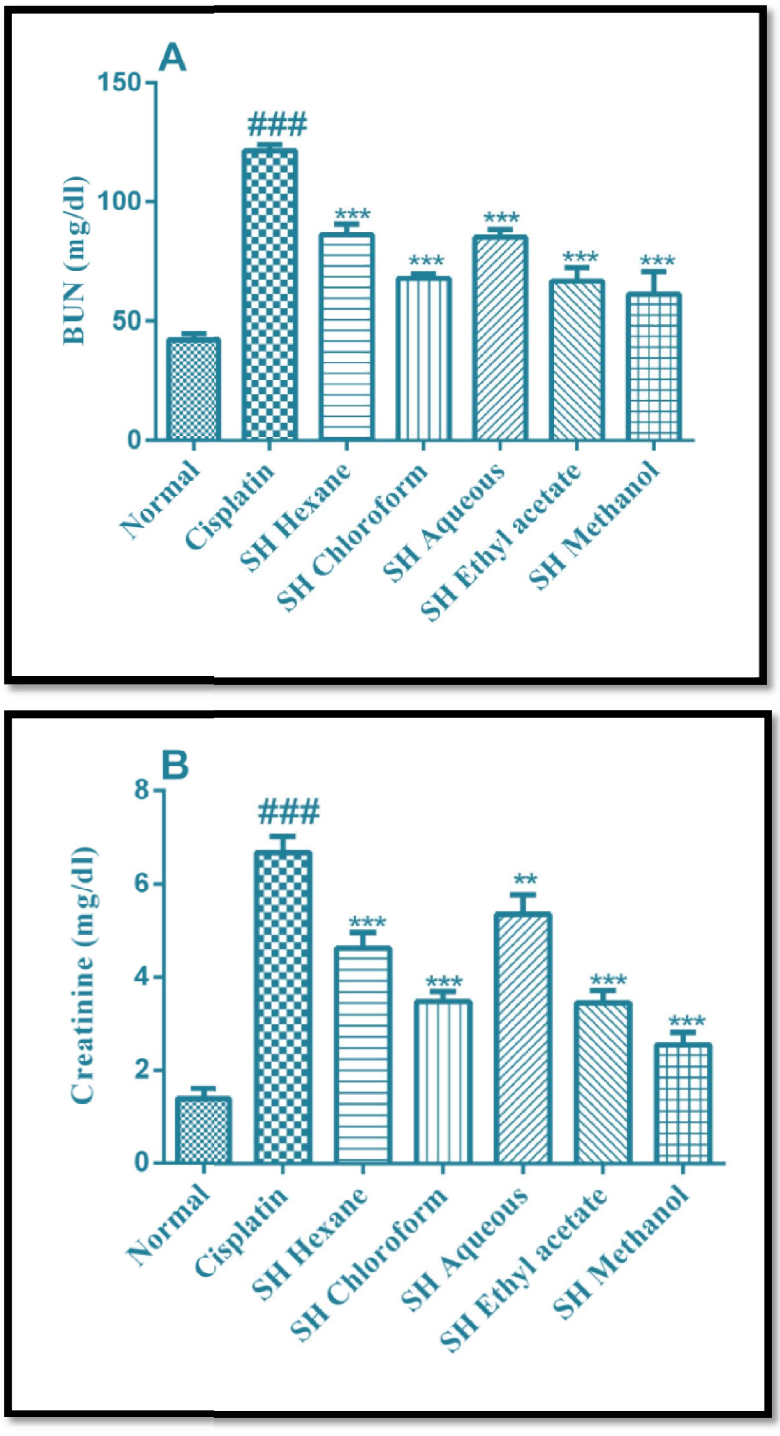

Figure 1: Effect of *S. hispida* extracts on A) BUN, B) Creatinine in Cisplatin-induced Nephrotoxicity rats

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

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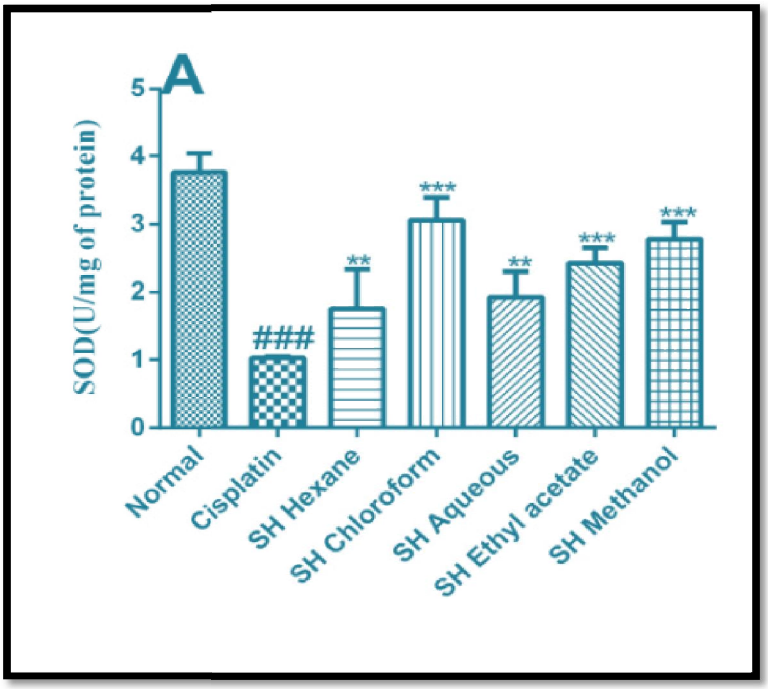
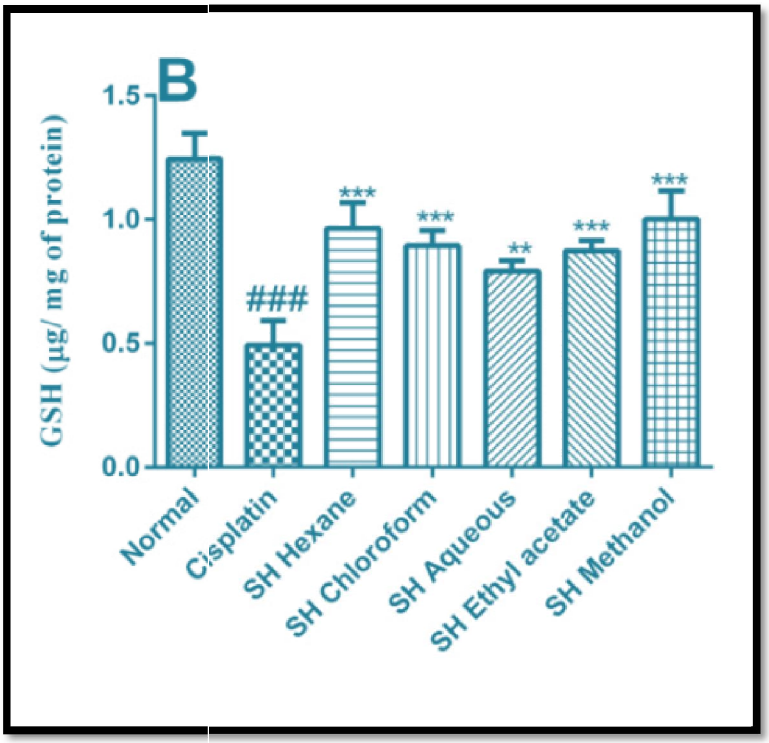
been a tremendous increase in GSH levels and the activities of SOD in the rats receiving *S. hispida* extract in different solvent shown in figure 2.





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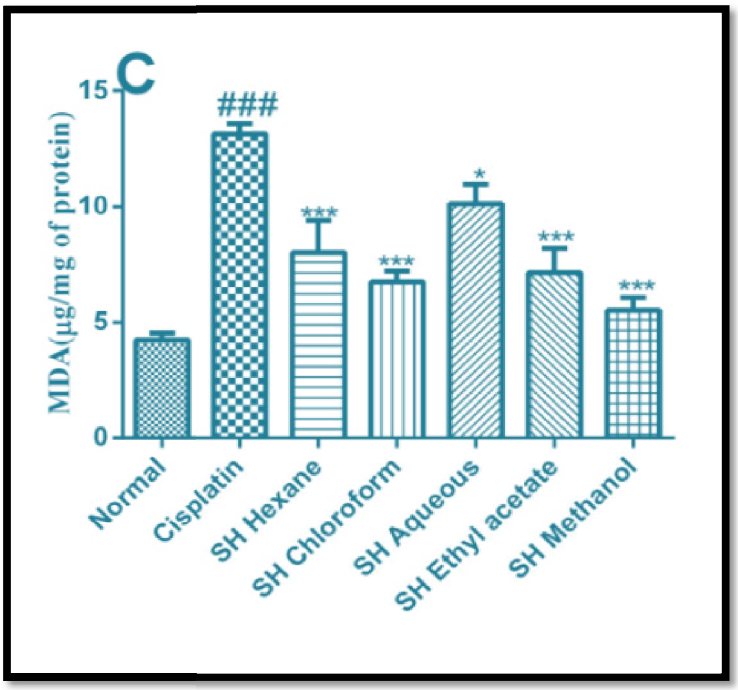
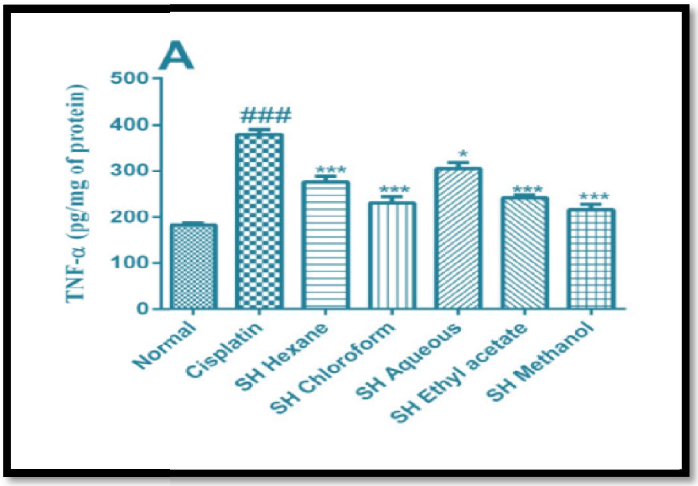


Figure 2: Effect of *S. hispida* extracts on A) SOD, B) GSH, C) MDA in Cisplatin-induced Nephrotoxicity rats

Effect of S. hispida on the cytokine release

We determined the levele of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β 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-1β 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.



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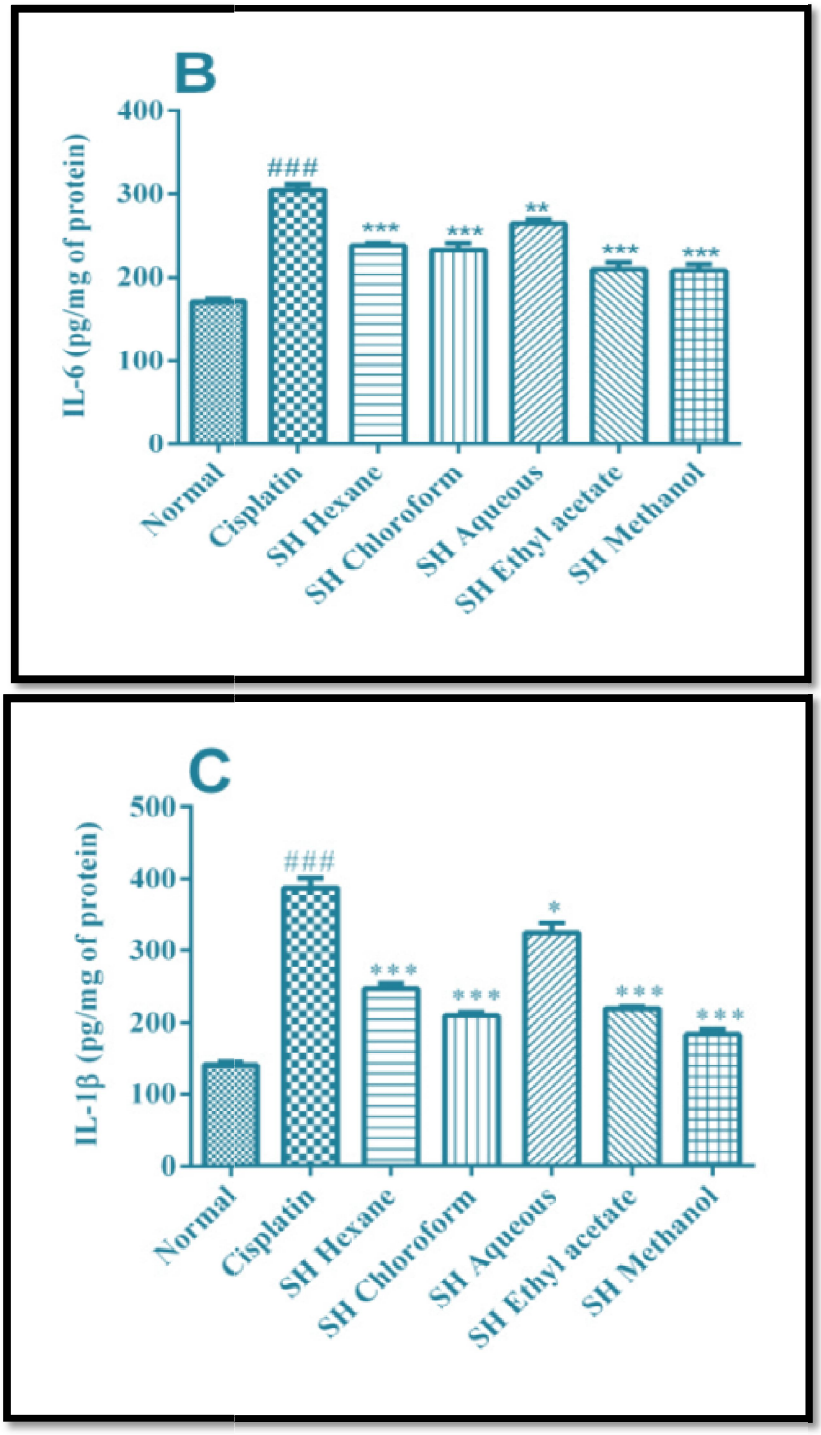

Figure 3: Effect of *S. hispida* extracts on A) TNF-alpha, B) IL-6, C) IL-1beta in Cisplatin-induced Nephrotoxicity rats

Effect of S. hispida on the Nitric oxide

Cisplatin treatment significantly increased the level of nitric oxide (505.2 ± 7.23 µM/mg) as compared to normal groups (235.5 ± 4.455 µM/mg) and Groups treated with *S. hispida* extract in different solvent at dose 100 mg/kg showed a

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decrease in nitric oxide level. Methanolic extract of *S. hispida* shows more potent activity than other solvent extract shown in figure 4.

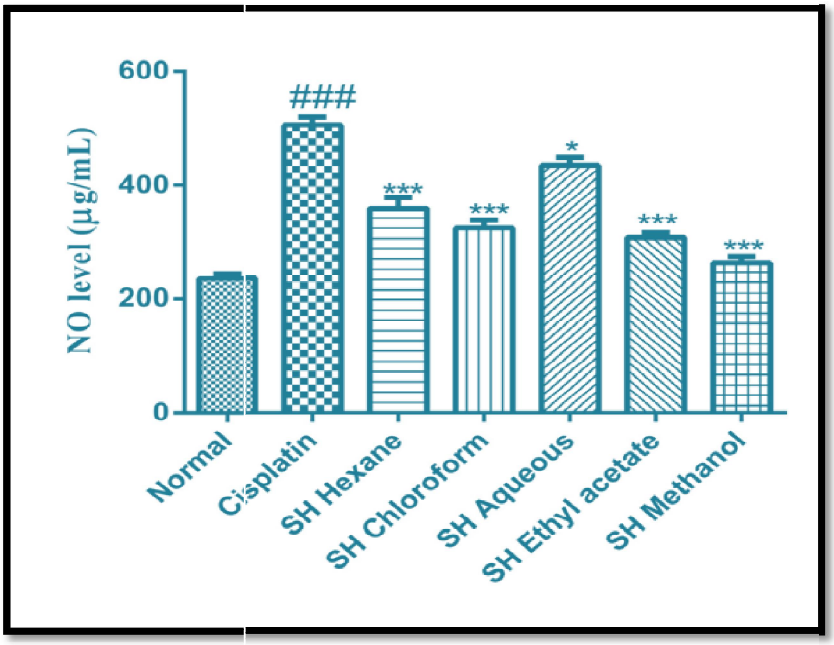

Figure 4: Effect of *S. hispida* extracts on nitric oxide in Cisplatin-induced Nephrotoxicity rats

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.

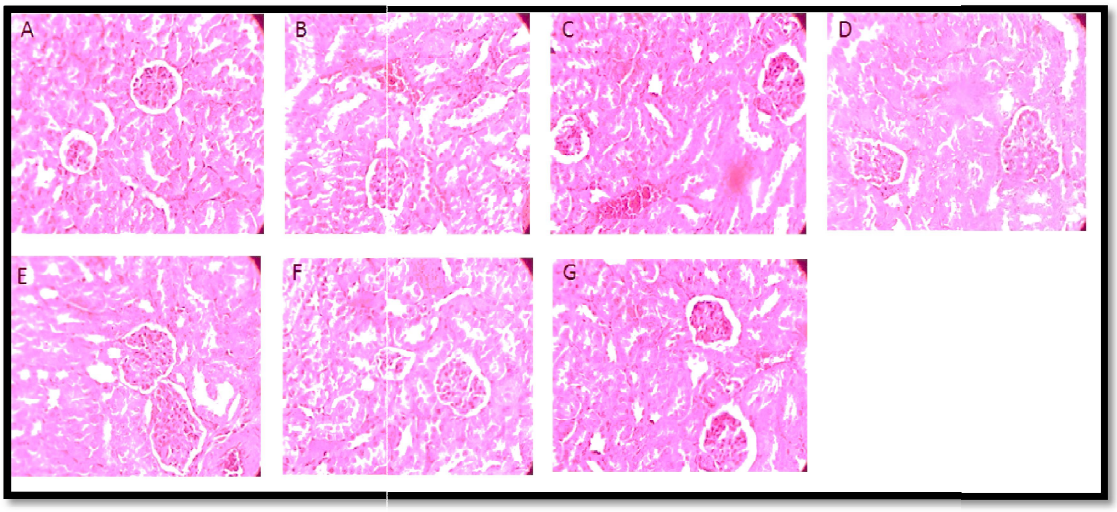

Figure 5: Effect of *S. hispida* extracts on histopathology of kidney in Cisplatin-induced Nephrotoxicity rats

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

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IV. DISCUSSION

Despite cisplatin's extensive usage as an antineoplastic agent, the drug's nephrotoxicity impact severely limits its therapeutic potential. About 40% of individuals get renal impairment as a result of cisplatin in cancer therapy after only one dosage [21]. No longer is it shown that S. hispida has a therapeutic impact on cisplatin-induced nephrotoxicity or the molecular mechanism by which it does so.

A variety of solvents, including chloroform, hexane, methanol, water, and ethyl acetate, were used to make extracts from S. hispida; the methanolic extract yielded the highest percentage. The plant was discovered to include alkaloids, flavonoids, carbs, proteins, phenols, steroids, and glycosides in the case of phytochemical screening.

In this work, we used experimental models to look into whether S. hispida may protect against cisplatin-induced nephrotoxicity.

As indicators of kidney impairment, BUN and serum creatinine may be used to diagnose cell injury. Injured tissue is likely to release these markers into the circulation when the cell membrane becomes more permeable or ruptures. Damaged renal tissue is the most typical cause of elevated levels of these markers of renal injury [22]. Blood urea nitrogen and serum creatinine levels were higher in rats that had been treated with cisplatin. The elevation of renal damage indicators was repressed by S. hispida treatment. Multiple investigations have linked reactive oxygen species (ROS)-induced oxidative damage to the development of nephrotoxicity [14]. The significance of oxidative stress in mitigating cisplatin-induced nephrotoxicity via S. hispida was highlighted in this investigation. After fourteen days of therapy with S. hispida, those symptoms disappeared. The pathophysiology of cisplatin-induced nephrotoxicity includes inflammation, which is similar to oxidative stress[23].

The classic inflammatory mediator tumor necrosis factor (TNF-) activates macrophages and neutrophils, which in turn causes irritation and the creation of cytokines, which in turn leads to cell necrosis. In addition to inducing inflammation and fever, IL-1 promotes the development and differentiation of the immune system [24]. S. hispida's inhibitory impact could help reduce inflammation caused by Cisplatin-induced loss of renal function and the overproduction of TNF-alpha, IL-6, and IL-1β.

Our results show that nitric oxide levels in Cisplatin-injected rats are greater than those reported in a number of other studies [25]. But when S. hispida was added to the mix, the nitric oxide production level turned back around. Symptoms of proximal tubule degeneration in renal tissue caused by cisplatin include hydropic degeneration, pycnotic nuclei, extended cytoplasmic vesicles, cytoplasmic vacuolization, necrosis, apoptosis, and desquamation of necrotic epithelial cells that fill the tubules[26]. Injecting rats with cisplatin had the same result. Damage to renal tissue was avoided in rats treated with S. hispida.

V. CONCLUSION

The study demonstrates that Spermacoce hispida exhibits significant nephroprotective effects against cisplatin-induced nephrotoxicity in an in vivo rat model. Treatment with S. hispida extracts resulted in a marked reduction in biomarkers of kidney damage, including blood urea nitrogen (BUN) and serum creatinine levels. Additionally, S. hispida treatment mitigated oxidative stress and lowered nitric oxide (NO) levels, which are critical factors in cisplatin-induced renal damage. Histological examinations confirmed that S. hispida effectively preserved kidney tissue structure compared to untreated cisplatin-exposed rats. These findings highlight the potential of Spermacoce hispida as a natural, protective agent against nephrotoxicity, offering a promising avenue for further research and potential therapeutic application.

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