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Research Article

INVESTIGATING THE NEPHROPROTECTIVE POTENTIAL OF *OKRA (ABELMOSCHUS ESCULENTUS)* AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Objective: Plants incorporated in the daily diet are known for their role in disease prevention owing to their diverse biological and pharmacological activities. The present study aimed to investigate the nephroprotective potential of hydro-alcoholic pod extract of *Okra* (*Abelmoschus esculentus*) against cisplatin-induced nephrotoxicity.

Methods: The chemical constituents of *Okra* extract were identified through preliminary phytochemical screening and liquid chromatography-mass spectrometry (LC-MS) spectral analysis. A total of 80 compounds were detected, including phytoconstituents with notable antioxidant properties such as alpha-linolenic acid, quercetin, myricitrin, quercetin-3-glucoside-7-xyloside, dioctyl hexanedioate, and protobassic acid. Nephroprotective activity was evaluated in albino Wistar rats at two dose levels (200 and 400 mg/kg b.w.). Nephrotoxicity was induced by a single intraperitoneal injection of cisplatin (6 mg/kg b.w.). The protective effects were assessed by estimating urinary total protein, creatinine clearance, serum creatinine (SC), blood urea nitrogen, lipid peroxidation, and antioxidant status.

Results: Nephrotoxicity arose in the group II rats due to cisplatin injections; according to this, SC and urea levels were significantly higher ($p<0.05$) when compared with the normal control group (I). Animals that received the hydroalcoholic extract of pods of *Okra* alone (group-V) exhibited no change in serum and urinary functional parameters. Animals that received the hydroalcoholic extract of pods of *Okra* alone (group-V) exhibited no change in serum and urinary functional parameters. Hence, the hydroalcoholic extract of pods of *Okra* did not show any nephrotoxic effects. Administration of hydroalcoholic extract of pods of *Okra* at 200 mg/kg to cisplatin-injected rats improved of blood urea and SC activity in treatment group III animals, when compared to cisplatin-induced group animals (group-II). It was practically near the normal values related with the normal group animals. On administration of hydroalcoholic extract of pods of *Okra* in treatment groups III and IV animals, a momentous dose-related reduction in the levels of blood parameters was detected when compared to the negative group-II animals.

Conclusion: The findings suggest that *Okra* hydro-alcoholic extract possesses strong nephroprotective activity, likely attributable to its antioxidant phytoconstituents. Hence, *Okra* may serve as a promising dietary intervention for mitigating cisplatin-induced nephrotoxicity.

Keywords: *Abelmoschus esculentus*, Liquid chromatography-mass spectrometry, Nephroprotective activity, Cisplatin, Hydro-alcoholic extract, Antioxidants and Wistar rats.

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INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum(II)) is a potent and frequently used chemotherapy agent [1]. Alone, or in combination with radiation, cisplatin is the first-line therapy for many malignancies including breast, testicular, ovary, cervical, head and neck, oral, bladder, prostate, gastric, small cell lung cancers, leukemia, lymphomas, neuroblastoma, and sarcomas. Yet, cisplatin treatment frequently causes detrimental side effects, the most serious effect being nephrotoxicity [2]. Acute kidney injury (AKI) caused by cisplatin therapy increases the risk of death of a person up to 15%, while 3-year mortality risk after AKI is 50% [3]. However, its use is limited by toxicity, including nephrotoxicity [4]. Plants vital for food and medicine are a growing area of interest for health benefits [5]. *Okra* (*Abelmoschus esculentus* L. (Moench) is one of the plants used as a vegetable and available throughout the year. It is the Malvaceae family (also known as *Hibiscus esculentus*), which is more commonly known in several other vernacular names as ladies finger, *Okra*, bhindi, or gumbo [6,7]. *Okra* contains carbohydrates, protein, fat, fiber, minerals, and vitamins [8]. In addition, *Okra* is a rich source of flavonoid compounds such as hyperoside, coumarin, scopoletin, hydroxycinnamic derivatives, oligomeric catechins, and flavonols that lead to its anti-oxidative characteristics [9]. (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging ability [10], antioxidant [11], α-amylase, and α-glucosidase inhibitory activities *in vitro*, hypoglycemic activity [12], anti-fatigue [13], antibacterial activity [14], and improved cell viability [15].

This study analyzes *Okra's* phytochemical profile using liquid chromatography-mass spectrometry (LC-MS) and explores its biological activities. Prior research indicates that *Okra* has various protective properties. LC-MS is an analytical technique that combines the separation power of liquid chromatography (LC) with the detection capabilities of mass spectrometry (MS), used for identifying, quantifying, and characterizing compounds in complex mixtures [16]. We hypothesized that the hydroalcoholic extract of *A. esculentus* pods would attenuate cisplatin-induced nephrotoxicity in rats through its antioxidant properties. The main objective of the present study is to evaluate the nephroprotective effect against cisplatin-induced toxicity.

METHODS

Chemicals

Cisplatin was purchased from Sigma Aldrich, Bangalore, India. Blood urea nitrogen (BUN) and serum creatinine (SC) kits were obtained from Diagnostics, Bangalore, India. All other chemicals were of analytical grade procured from the Empire Scientific Company, Andhra Pradesh.

Collection, authentication, and preparation of plant extract

The pods of *Okra* were collected from the local market, Bapatla, district of Bapatla, Andhra Pradesh. The plant was authenticated by Botanist Dr. Raju, Herbarium keeper, Department of Botany, Acharya Nagarjuna University, Guntur, India.

The *Okra* pods were washed with distilled water, cut into pieces, and dried in the shade. One kilogram of dried *Okra* pods was produced 200 g powdered using mixer. After defatting with pet ether (60–80°C), the yield of the product is 180 g. The defatted marc was air dried and subjected to soxhlation with hydroalcoholic solution (1:1) for 72 h using rotary evaporation under reduced pressure. The yield of the product was 20%. The collected hydroalcoholic extract was characterized using preliminary phytochemical screening using standard procedures to identify the presence of various phytoconstituents [17] and LC-MS study.

Animals

Wistar albino rats, both sexes (body weight 150–200 g), were purchased from Mahaveer Enterprises, Hyderabad, India, and were kept under standard conditions of temperature and humidity in the center's animal house facility. The animals were kept on a standard rat diet and water *ad libitum.* In this, the animal experimental studies were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) (CPCSEA approval No. IAEC/XVI/04/BCOP/2023).

Experimental design

Screening of nephroprotective activity

Animals were divided into five groups (n=6). The cisplatin at a dose of 6 mg/kg b.w through single intraperitoneal administration on day 5th was used to induce nephrotoxicity. The treatment schedule was mentioned in Table 1.

The treatment schedule was followed:

The extract was given as a suspension (the weighed extract+water) form to the animals from day 1 to day 10; the cisplatin was given on the 5th day to all groups of animals except group-I. The group-V animals received the high dose of extract (400 mg/kg) for 10 days without cisplatin injection. On day 9, urine was collected with the help of metabolic cages and the urine samples were subjected to estimation of urinary functional parameters. The animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture, and serum was separated by centrifugation at 3000 rpm at 4°C, were used for estimation of serum markers. For estimation of BUN, SC the serum is used, that serum was separated using centrifuge process at 3000 rpm, at the temp 4°C. Then, the animals were sacrificed by cervical decapitation and both kidneys were harvested. One of the kidneys was used for antioxidant studies and other was subjected to histological examination.

Assessment of renal function

Urinary and serum parameters

Urinary and renal functions were assessed by measuring biochemical parameters. The urinary total protein (turbidity method) and urinary creatinine (alkaline picrate method), renal functions such as BUN (DAM method), SC (Jaffe's Alkaline picrate method), serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alanine aminotransferase (ALP) were estimated using commercial kits in semi-automatic analyzer [9].

Renal oxidative stress markers

Weighed portions of the tissues were homogenized in ice-cold 0.05 M phosphate buffer pH 7.8 to obtain a 20% (w/v) homogenate as described by Aksnes and Njaa (1981). The homogenates were centrifuged at 3000 rpm for 15 min and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes.

Statistical analysis

Values were represented as mean ± SEM. Data were analyzed using one-way analysis of variance. $p<0.05$ was considered significant.

RESULTS

Yield and preliminary phytochemical screening

The yield of hydroalcoholic extract of pods of *Okra* was 20%.

The preliminary phytochemical screening of the hydroalcoholic extract of *Okra* revealed the presence of carbohydrates, flavonoids, saponins, phenolic compounds, vitamins, oligomeric catechins, volatile oils/ essential oils, and flavonol glycosides.

LC/MS analysis

LC-MS/MS chromatogram (Fig. 1) analysis of *Okra* revealed a rich profile of compounds mentioned in Table 2. It reveals the presence of the N-acetylglucosamine, 10-acetoxyligustroside, debromohymenialdisine, 6-sialyllactose, sarmentosin epoxide, and all other substances. These identified active compounds suggest a potential link to the plant's nephroprotective capabilities, with the comprehensive analysis confirming the presence of all tested standards.

Effect of hydroalcoholic extract of *Okra* on serum and urinary parameters

Nephrotoxicity arose in the group II rats due to cisplatin injections; according to this, SC and urea levels were pointedly higher ($p<0.05$) when compared with the normal control group (I). Animals which received the hydroalcoholic extract of pods of *Okra* alone (group-V) exhibited no change in serum and urinary functional parameters. Hence, the hydroalcoholic extract of pods of *Okra* did not show any nephrotoxic effects. Administration of hydroalcoholic extract of pods of *Okra* at 200 mg/kg to cisplatin-injected rats improved blood urea and SC activity in treatment group III animals, when compared to cisplatin-induced group animals (group-II). It was practically near the normal values related to the normal group animals. On administration of hydroalcoholic extract of pods of *Okra* in treatment groups III and IV animals, a momentous dose-related reduction in the levels of blood parameters was detected when compared to the negative group-II animals. The decline of renal functions brought by cisplatin and the result of oral administration of the hydroalcoholic extract of pods of *Okra* are given in Table 3.

DISCUSSION

The present study demonstrated that the hydroalcoholic extract of *A. esculentus* pods exerts significant nephroprotective effects against cisplatin-induced nephrotoxicity in Wistar albino rats. The key findings were a marked reduction in SC, BUN, and urinary protein levels, along

Table 1: Treatment schedule

S. No.	Group	Treatment	Dose	No. of days of treatment
1	Group-I	Normal saline	0.9% v/w	10 days
2	Group-II	Cisplatin	6 mg/kg b.w.	On 5th day
3	Group-III	Extract-low dose	200 mg/kg b.w	1–10 days, the cisplatin injected on 5th day
4	Group-IV	Extract-high dose	400 mg/kg b.w	1–10 days, the cisplatin injected on 5th day
5	Group-V	Only extract	400 mg/kg b.w	1-10 days

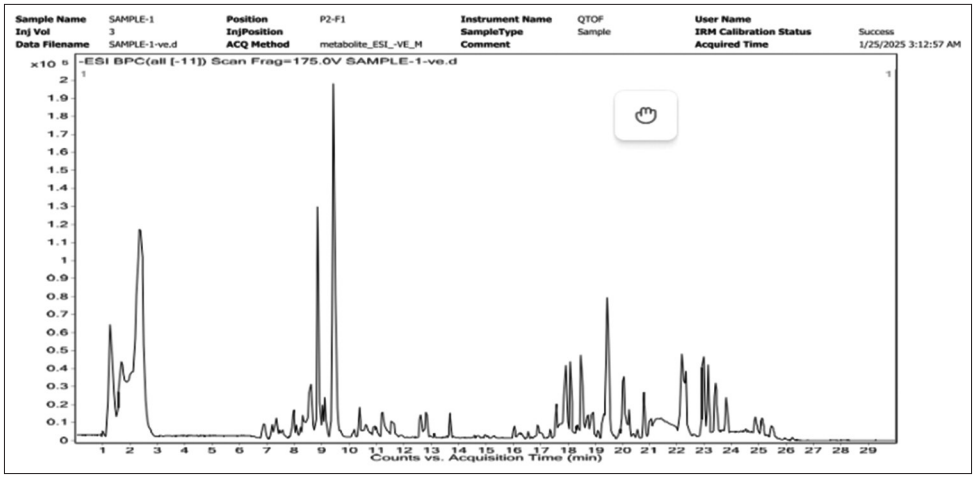

Fig. 1: High-resolution liquid chromatography-mass spectrometry chromatogram of hydroalcoholic extract of *Okra*

Table 2: LC-MS study of hydroalcoholic extract of *Okra*

Compound Label	RT	Mass	Name	Formula
Compound: 2	1.174	235.07	N-Acetylgalactosamine	$C_8H_{13}NO_7$
Compound-29	9.01	596.1397	Quercetin3-glucoside7-xyloside	$C_{26}H_{28}O_{16}$
Compound-33	9.496	464.0976	Myricitrin	$C_{21}H_{20}O_{12}$
Compound-46	12.577	580.3474	Alpha, alpha'-trehalose 6-palmitate	$C_{28}H_{52}O_{12}$
Compound-67	19.354	340.2081	Canrenone	$C_{22}H_{28}O_3$
Compound-72	20.862	448.2847	16-Feruloyloxypalmitate	$C_{26}H_{40}O_6$
Compound-75	22.075	562.2605	19-Hydroxycinnzeylanol19-glucoside	$C_{26}H_{42}O_{13}$
Compound-76	22.359	370.3092	Dioctylhexanedioate	$C_{22}H_{42}O_4$
Compound-78	22.975	300.2676	2S-hydroxy-octadecanoic acid	$C_{18}H_{36}O_3$
Compound-79	23.729	504.3468	Protobassic acid	$C_{30}H_{48}O_6$

with an increase in creatinine clearance. These parameters are direct indicators of improved renal function, suggesting that *Okra* extract effectively mitigates cisplatin-induced renal damage.

Nephrotoxicity is one of the major side effects in the course of chemotherapy with various drugs [18,19]. Cisplatin is one of the best and first metal-based chemotherapeutic drugs as a potent anticancer drug and is unfortunately associated with significant nephrotoxicity, a major factor limiting its clinical use [20]. Cisplatin binds to DNA and forms inter- and intra-strand crosslinks, thus arresting DNA synthesis and replication. The kidney accumulates cisplatin to a greater extent unlike other organs and is the major route for its excretion. The Cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity, resulting in oxidative stress, evident from elevated lipid peroxidation (LPO) and depleted levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) and triggers these damaging processes. Cisplatin in the kidneys penetrates tubular cells and concentrates mainly in the proximal tubules, causing tubular damage. Tubular damage is characterized by a reduced glomerular filtration rate, increased SC, and BUN. Toxins that cause tubular injury share many pathophysiological features with ischemic damage. The resulting injury can severely compromise kidney function [21].

The compounds that were capable of minimizing nephrotoxic effects are called nephroprotectors [22]. The plants have various complex chemical substances such as flavonoids and saponins, which may help to protect against cisplatin-induced kidney damage due to their antioxidant, anti-inflammatory, and anti-apoptotic properties [23].

Okra (*A. esculentus*) is one of the medicinal plants with a rich source of polyphenolic compounds, flavonoids, and vitamins [24]. Compound exhibits a wide range of beneficial properties, including antioxidant, anti-inflammatory, immunomodulatory, antibacterial, anticancer, antidiabetic, organ-protective, and neuropharmacological effects [25].

The aim of this research work was to determine the potential activity of the hydroalcoholic extract of pods of *A. esculentus* against cisplatin-induced nephrotoxicity in Wistar albino rats. Effects of hydroalcoholic extracts of *Okra* were tested at two dose levels, i.e., 200 and 400 mg/kg body weight p.o against cisplatin-induced nephrotoxicity. Nephroprotective activity was assessed by determination of serum marker levels and urinary functional parameters, liver markers, and determination of antioxidant enzyme activities such as LPO, GSH, SOD, and CAT. Animals which received plant extracts alone for 10 days did not cause any change in serum markers, urinary functional parameter levels, and antioxidant enzyme levels when compared to normal control animals. These results suggest that hydroalcoholic extracts of *Okra* did not show any deteriorative effect on the kidney [26].

Cisplatin (6 mg/kg, i.p) as a single dose given to the rats resulted in recognizable nephrotoxicity was observed. Cisplatin treatment resulted in kidney damage, evident from elevated SC and BUN, increased urinary protein, and reduced creatinine clearance. This damage is likely due to impaired renal function and increased vascular resistance, consistent with prior research [27]. The cisplatin produced elevated levels were reversed in treatment groups, when compared the high dose treatment group showed more protective effect than low dose treatment group due to the presence of flavonoids [28].

The levels of SGPT, SGOT, and ALP in the serum were also found, and it was found that administration of cisplatin produced a significant increase in the levels of SGPT, SGOT, and ALP, respectively, as compared to the normal group rats (Table 4). It was observed that SGPT level was effectively reduced by both selected doses of hydroalcoholic extract of pods of *Okra*; however, high dose (400 mg/kg) produced a highly significant reduction in SGOT, SGPT, and ALP levels as compared to low dose 200 mg/kg. The results were consistent with earlier reports [29].

Cisplatin alone can significantly increase the antioxidant enzymes of LPO levels and decrease the levels of CAT, SOD, and GSH values depicted in Table 5; these findings agree with the previous reports [30]. Both doses were significantly reversed all the effects induced by cisplatin and the effect is dose dependent. At dose of 200 mg/kg body weight, the extract showed significant but moderate protection whereas at

Table 3: Effect of hydroalcoholic extract of *Okra* on serum and urinary parameters

Group	BUN (mg/dL)	SC (mg/dL)	U_{TP} (mg/24 h)	Creatinine clearance (mL/min)
Group-I-Normal saline	16.25±0.25	0.62±0.02	3.12±0.28	24.21±1.56
Group-II cisplatin	39.23±0.21[a]	3.31±0.08[a]	7.69±0.23[a]	5.43±0.45[a]
Group-III extract-low dose	29.01±1.08[b]	0.70±0.02[b]	5.42±0.44[b]	12.02±0.36[b]
Group-IV extract-high dose	24.01±1.37[b]	0.56±0.04[b]	4.25±0.31[b]	15.06±1.42[b]
Group-V only extract	18.25±0.22	0.68±0.02	3.98±0.24	20.60±1.02

Each value represents mean±SEM of six animals in each group. [a]p<0.05 when compared with Group-I; [b]p<0.05 when compared with Group-II, BUN: Blood urea nitrogen

Table 4: Effect of hydroalcoholic extract of *Okra* on liver parameters

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group-I-Normal Saline	35.83±1.49	31.17±1.70	46.33±2.40
Group-II cisplatin	152.1±7.07[a]	135.8±7.41[a]	291.5±15.45[a]
Group-III extract-low dose	126.1±5.94[b]	114.9±3.83[b]	240.8±17.27[b]
Group-IV extract-high dose	79.00±3.37[b]	69.47±3.13[b]	99.67±2.33[b]
Group-V only extract	31.83±1.49	29.17±1.70	42.33±2.40

Each value represents mean±SEM of six animals in each group. [a]p<0.05 when compared with Group-I; [b]p<0.05 when compared with Group-II

Table 5: Effect of hydroalcoholic extract of *Okra* on renal oxidative stress markers

Group	LPO (nmol/g of tissue)	GSH (nmol/g of tissue)	SOD (units/mg of tissue)	CAT (units/mg of tissue)
Group-I-normal saline	3.20±0.02	1.45±0.04	35.00±0.61	32.12±0.72
Group-II Cisplatin	8.12±0.25[a]	0.48±0.02[a]	10.23±0.36[a]	12.43±1.19[a]
Group-III extract-low dose	5.48±0.46[b]	0.68±0.02[b]	16.02±0.40[b]	19.76±1.11[b]
Group-IV extract-high dose	4.28±0.27[b]	0.81±0.01[b]	19.86±0.82[b]	24.76±0.97[b]
Group-V only extract	3.48±0.19	0.98±0.03	29.20±1.35	29.46±1.13

Each value represents mean±SEM of six animals in each group. [a]p<0.05 when compared with Group-I; [b]p<0.05 when compared with Group-II, LPO: Lipid peroxidation, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione

dose of 400 mg/kg body weight showed significant protection against cisplatin-induced nephrotoxicity.

Several previous studies have reported the nephroprotective effects of medicinal plants possessing strong antioxidant properties. Kiruba *et al*. demonstrated the nephroprotective efficacy of *Cynodon dactylon* and *Gmelina* species, while Yadav *et al*.[21] reported that the standardized hydroalcoholic extract of *Asparagus racemosus* effectively attenuated cisplatin-induced nephrotoxicity in Wistar rats. These studies support the concept that phytochemicals with antioxidant and anti-inflammatory activities play a pivotal role in renal protection [31,32].

In line with these reports, the present investigation confirms that the hydroalcoholic extract of *A. esculentus* (*Okra*) also exhibits significant nephroprotective activity. Treatment with *Okra* extract restored the balance of these antioxidant markers in a dose-dependent manner. The restoration of antioxidant defense is consistent with the LC-MS profile of the extract, which revealed the presence of flavonol glycosides such as quercetin derivatives and myricitrin. These compounds are well-documented free radical scavengers and are likely to have contributed to the observed renoprotective activity through their antioxidant, anti-inflammatory, and anti-apoptotic properties [33,34].

CONCLUSION

The present study demonstrates that the hydroalcoholic extract of Okra (Abelmoschus esculentus) pods exhibits significant nephroprotective potential against cisplatin-induced nephrotoxicity in Wistar rats. The protective effect can be attributed to the presence of potent phytoconstituents such as quercetin, myricitrin, and alpha-linolenic acid, which possess strong antioxidant and free radical scavenging properties. Administration of the Okra extract notably restored renal function markers, reduced oxidative stress, and preserved antioxidant enzyme levels, particularly at the 400 mg/kg dose. Therefore, Okra pods represent a promising natural therapeutic agent for mitigating cisplatin-induced renal damage and oxidative stress–related disorders.

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AUTHOR CONTRIBUTIONS

Conceptualization and Design: Prof. KVSRG Prasad, Research Supervision of Project: Dr. Sujatha, Experimentation work and writing: G. Swarupa Rani

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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