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Research Article

# ALLEVIATION OF CISPLATIN-INDUCED NEPHROTOXICITY IN ALBINO RATS BY ROOTS OF *CATUNAREGAM ULIGINOSA*

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**ABSTRACT**

**Objectives:** To evaluate the nephroprotective effect of ethanolic extract of roots of *Catunaregam ulginosa* (EECU) in cisplatin-induced nephrotoxicity in albino rats.

**Methods:** Ethanolic extract was prepared by hot extraction method and subjected to preliminary phytochemical studies. Based on acute toxicity studies, nephroprotector was screened at two dose levels, i.e., 200 mg/kg bd. Wt. and 400 mg/kg bd. Wt. in both curative and prophylactic regimens. Cisplatin at a dose of 5 mg/kg bd. Wt. was given intraperitoneally to induce nephrotoxicity. Nephroprotector activity was assessed by determining blood urea nitrogen, serum creatinine, urinary total protein ($U_{TP}$), and creatinine clearance (Clcr). Antioxidant enzymes such as super oxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH) activities, and lipid peroxidation (LPO) levels were determined in renal tissue. Histological studies had been carried *per se*.

**Results:** Animals that received cisplatin exhibited a significant increase in serum marker levels, increased $U_{TP}$ excretion, and reduced Clcr. Further significant decrease in GSH, SOD, CAT, and increase in LPO levels was observed. EECU reversed the effects induced by cisplatin in a dose-dependent manner in both curative and prophylactic regimens. Histological studies substantiated the above results.

**Conclusion:** The findings of the present study support the alleviation of cisplatin-induced nephrotoxicity by the EECU and thus validate its ethnomedicinal use.

**Keywords:** *Catunaregam ulginosa*, Cisplatin, Nephrotoxicity, Lipid peroxidation.

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## INTRODUCTION

Medicinal plants are the potential source of therapeutic aid for the treatment and prevention of various ailments from ancient times [1]. According to an analysis by the WHO, over 80% of the world's population relies on traditional forms of medicine, largely plant based to meet their primary health-care needs [2]. There have always been counting emphases on the herbal medicines as a potential pipeline for novel bioactive molecules. The rich biodiversity of plants makes them a treasure house for obtaining new and novel compounds either themselves as drugs or lead molecules for drugs with different mechanisms of action. Medicinal plants contain several different phytochemicals or secondary metabolites that may act individually, additively or in synergy to improve health [3]. The different plant extracts have different modes of action for curing diseases. The therapeutic efficacy of plants is because of the phytochemicals which include alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, and tannins. Many medicinal plants have been scientifically evaluated and found to possess various activities such as antidiabetic, wound healing, analgesic, anti-inflammatory, antineoplastic, antiobesity, and nephroprotector efficacy.

Nephrotoxicity is a renal disease or dysfunction which arises mainly due to exposure to medicines, industrial/environmental chemicals, age, or pre-renal diseases. Drugs are being common source of nephrotoxicity. Drug-induced nephrotoxicity has become more common among certain clinical situations such as chemotherapy, wide use of non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, antimicrobial agents [4] drugs (such as cisplatin, gentamicin, doxorubicin), and toxic chemicals (such as cadmium and lead) are some of the substances which result in nephrotoxicity.

Cisplatin (*cis*-diamminedichloroplatinum (II)), one of the most commonly used antineoplastic agents induces nephrotoxicity. It is widely used in the treatment of many solid-organ cancers, including those of the head, neck, lung, testis, ovary, and breast. The toxicities of cisplatin include ototoxicity, gastrotoxicity, myelosuppression, nephrotoxicity, and allergic reactions [5,6]. The main dose-limiting side effect is nephrotoxicity which was experienced in about 25-35% of patients after receiving a single dose of cisplatin [7]. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity [8].

Various medicinal plants had been used in traditional medicine and by folklore for the treatment of renal diseases. *Catunaregam uliginosa* (Family: Rubiaceae) is one such plant roots of which were used by folklore of Rayalseema for the treatment of urinary problems such as strangury, dysuria and also as a diuretic and cooling agent [9]. However, until today, there was no scientific evidence for the ethnopharmacological use of this plant as a nephroprotector agent. Hence, the present study was aimed to evaluate the nephroprotector activity of ethanol extract of roots of *Catunaregam uliginosa* (EECU) against cisplatin-induced nephrotoxicity.

## METHODS

### Collection of plant material

Roots of *C. uliginosa* were collected from Tirupati, Chittor Dt., Andhra Pradesh and authenticated by botanist Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati, and a voucher specimen was deposited in S.V. University, Department of Botany, Tirupati.

### Preparation of extract

Roots of *C. uliginosa* were thoroughly washed, shade dried, and powdered in a Wiley mill. The powdered roots were defatted using

petroleum ether (60-80°C). The defatted marc was air-dried and macerated with ethanol for 24 hrs. Macerated material was refluxed for 3 hrs, then filtered and subjected to distillation under reduced pressure.

**Preliminary phytochemical screening**

The EECU was subjected to preliminary phytochemical studies for the presence of various phytoconstituents such as alkaloids, amino acids, saponins, flavonoids, glycosides, steroids, tannins, terpenoids, carbohydrates, fixed oils, and fats [10].

**Pharmacological studies**

*Animals*

Healthy Wistar strain male adult albino rats between 2 and 3 months of age and weighing about 150-200 g had been used in the present study. They were maintained in a 12 hrs light/dark cycle at a constant temperature 25°C with free access to standard rat pellet diet and water *ad libitum*. All experiments were carried out according to the guidelines for care and use of experimental animals by committee for the purpose of control and supervision of experiments on animals. This study was approved by the Institutional Animal Ethical Committee.

**Acute oral toxicity studies**

Oral acute toxicity studies were conducted for EECU at 2000 mg/kg body weight as per Organization for Economic Cooperation and Development Guidelines No. 423 [11]. Any changes in body weights of the rat, changes in skin and fur, eyes and mucous membranes, salivation, nasal discharge, urination and behavioral (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular changes, lethargy, sleep, and coma were observed. The animals were kept under observation for 14 days.

**Treatment protocol**

Animals were divided into eight groups of six each. Nephrotoxicity was induced by single intraperitoneal administration of cisplatin at a dose of 5 mg/kg bd. wt. The following treatment schedule was followed:

- Group-I: Vehicle (10 mg/kg bd. wt.) from day 1 to 5.
- Group-II: Curative control-cisplatin (5 mg/kg, i.p.) on day 1+ vehicle from day 5 to 9.
- Group-III: Prophylactic control-vehicle from day 1 to 5 + cisplatin (5 mg/kg, i.p.) on day 5.
- Group-IV: Curative effect-cisplatin (5 mg/kg, i.p.) on day 1 + EECU 200 mg/kg bd. wt. from day 5 to 9.
- Group-V: Curative effect-cisplatin (5 mg/kg, i.p.) on day 1+ EECU 400 mg/kg bd. wt. from day 5 to 9.
- Group-VI: Prophylactic effect-EECU 200 mg/kg bd. wt. from day 1 to 5 + cisplatin (5 mg/kg) on day 5.
- Group-VII: Prophylactic effect-EECU 400 mg/kg bd. wt. from day 1 to 5 + cisplatin (5 mg/kg) on day 5.
- Group-VIII: Only higher dose of the extract from day 1 to 5.

**Biochemical determination**

On day 5 from animals of Groups-I and VIII and on day 10 from remaining groups, urine was collected with the help of metabolic cages, and the urine samples were subjected for estimation of urinary functional parameters. The animals were sacrificed on the respective following days by cervical decapitation, and blood samples were collected by cardiac puncture and were used for estimation of serum markers. Kidneys were isolated and used for antioxidant and histopathological studies.

**Assessment of nephroprotector activity**

Nephroprotector activity was assessed by estimating blood urea nitrogen (BUN) by diacetyl monoxime method, serum creatinine (SC) by Jaffe's alkaline picrate method, urinary total proteins ($U_{TP}$) by turbidimetry method, and creatinine clearance (Clcr) by alkaline picrate method and was calculated using formula Clcr = Urinary creatinine x urinary volume/hr/SC [12].

**Antioxidant studies**

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. The homogenate was centrifuged at 10,000 rpm for 15 minutes, and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes. Antioxidant studies were carried out by the estimation of levels of reduced glutathione (GSH), catalase (CAT), superoxide dismutase, and lipid peroxidation (LPO) [13-16].

**Histological studies**

Kidneys of two animals from each group were used for histological studies. The isolated kidneys were fixed in 10% neutral buffer formalin and processed to paraffin wax. Sections (5 microns) were stained with hematoxylin and eosin and are examined under light microscope.

**Statistical analysis**

The statistical data were presented as mean±standard error of mean. Parametric data, which include all the biochemical parameters, were analyzed using one-way analysis of variance followed by a Dunnett's multiple comparisons post-test. A probability value of $p \leq 0.05$ was considered as significant.

**RESULTS**

**Preliminary phytochemical studies**

Preliminary phytochemical studies showed the presence of alkaloids, glycosides, saponins, triterpenoids, steroids, tannins, carbohydrates, gums, and mucilages.

**Acute oral toxicity studies**

Acute toxicity studies revealed that the EECU is safe up to 2000 mg/kg bd. wt.

**Evaluation of nephroprotector activity**

*Effect on serum and urinary parameters*

Administration of EECU alone for 6 days did not show any deteriorative effects on kidney revealing that the extract is safe. Cisplatin injected animals exhibited a significant increase in BUN, SC, $U_{TP}$, and decreased Clcr. Animals treated with ethanolic extract at 200 and 400 mg/kg bd. wt. reversed the effects induced by the cisplatin in the dose-dependent manner in both curative and prophylactic regimens (Table 1).

**Table 1: Effect of ethanol extract of *C. uliginosa* on renal functional parameters**

| Group | BUN (mg/dl) | SC (mg/dl) | $U_{TP}$ (mg/24 hrs) | $Cl_{cr}$ (ml/hr/100g bd. wt.) |
|---|---|---|---|---|
| I | 9.83±1.47 | 1.13±0.19 | 2.35±0.35 | 11.86±0.77 |
| II | 31.83±2.78*** | 3.06±0.53*** | 5.42±0.76*** | 2.44±0.73*** |
| III | 33.50±2.42*** | 2.70±0.45*** | 5.31±0.47*** | 2.28±0.54*** |
| IV | 15.33±2.33*** | 2.05±0.30** | 2.25±0.36*** | 6.89±1.11*** |
| V | 11.17±2.22*** | 1.23±0.34*** | 1.40±0.67*** | 10.28±1.72*** |
| VI | 14.83±1.94*** | 1.81±0.39* | 2.10±0.18*** | 7.08±1.63*** |
| VII | 13.50±3.01*** | 1.33±0.47*** | 1.35±0.62*** | 9.57±2.040*** |
| VIII | 10.67±2.58 | 1.36±0.35 | 2.12±0.062 | 10.71±1.73 |

Each value represents the mean±SEM from 6 animals in each group. ***: p<0.0001; **: p<0.001; *: p<0.05. Groups-II and III compared with Group-I; Groups-IV and V compared with Group-II; Groups-VI and VII compared with Group-III. SEM: Standard error of the mean, *C. uliginosa: Catunaregam uliginosa*, BUN: Blood urea nitrogen, SC: Serum creatinine, $U_{TP}$: Urinary total protein, $Cl_{cr}$: Creatinine clearance

**Table 2: Effect of ethanol extract of *C. uliginosa* on antioxidant levels against cisplatin-induced nephrotoxicity**

| Group | LPO (nm/100 mg of tissue) | SOD (units/mg of tissue) | CAT (units/mg of tissue) | GSH (nm/100 mg of tissue) |
|---|---|---|---|---|
| I | 2.050±0.2389 | 27.79±5.468 | 11.21±3.711 | 26.50±1.416 |
| II | 4.380±0.8734*** | 13.05±1.815*** | 4.215±1.243** | 14.37±1.083*** |
| III | 5.553±0.6107*** | 13.99±2.736*** | 4.294±1.162** | 14.01±1.714*** |
| IV | 2.165±0.2584*** | 23.18±4.988* | 8.622±1.926* | 19.74±1.239*** |
| V | 1.122±0.2198*** | 27.06±5.257*** | 10.48±3.839** | 24.71±1.373*** |
| VI | 2.143±0.2497*** | 22.82±4.921* | 9.001±2.327* | 18.41±1.471** |
| VII | 1.189±0.1883*** | 27.38±4.939** | 10.05±2.821* | 22.26±2.146*** |
| VIII | 1.960±0.2476 | 27.81±5.711 | 11.33±3.326 | 25.73±2.592 |

Each value represents the mean±SEM from 6 animals in each group. ***: p<0.0001; **: p<0.001; *: p<0.05. Groups-II and III compared with Group-I; Groups-IV and V compared with Group-II; Groups-VI and VII compared with Group-III. SEM: Standard error of the mean, *C. uliginosa: Catunaregam uliginosa*, LOP: Lipid peroxidation, SOD: Superoxide dismutase, CAT: Catalase, GHS: Glutathione

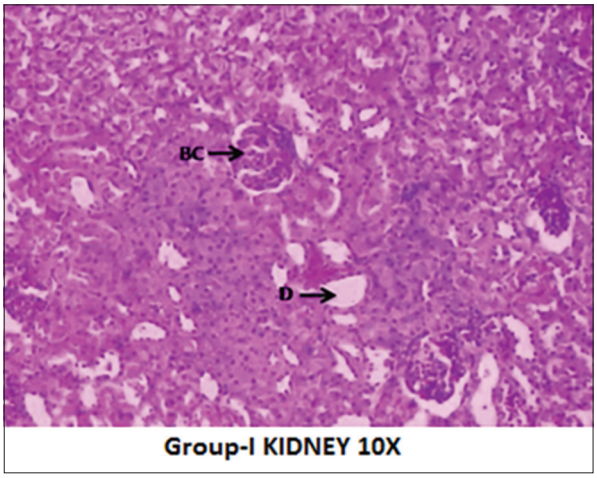


**Fig. 1: Section of normal rat kidney showing normal organization of tubular epithelial cells and glomeruli**

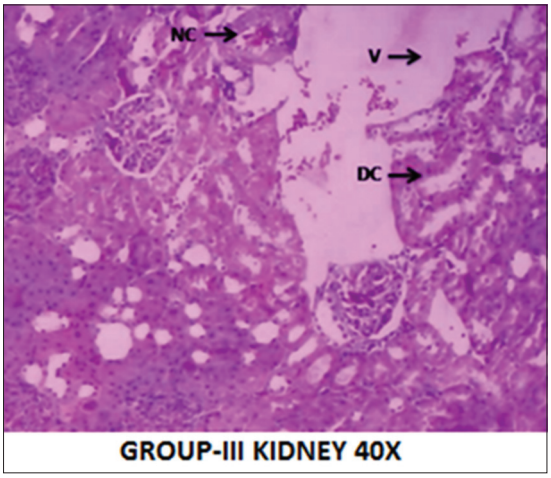


**Fig. 3: Section of rat kidney showing severe congestion in glomeruli, vacuolization, and necrotic changes (prophylactic control)**

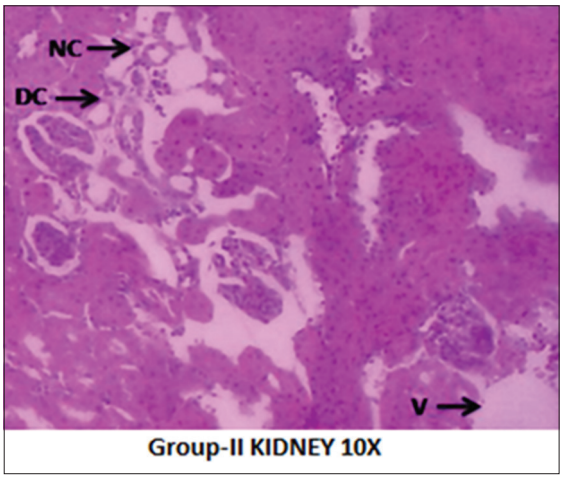


**Fig. 2: Section of rat kidney showing necrotic changes in kidney tissues and congestion with hemorrhages (curative control)**

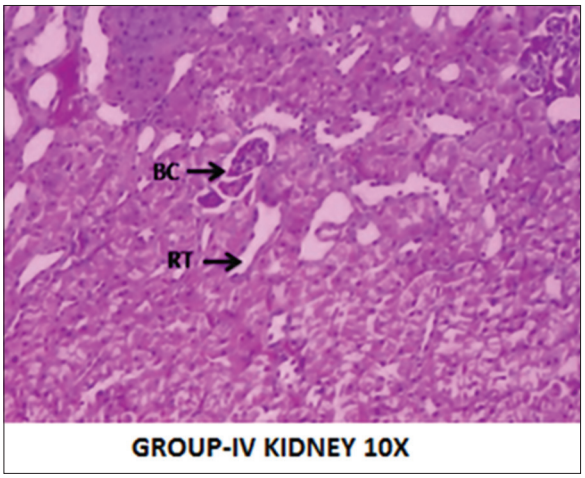


**Fig. 4: Section of rat kidney showing mild regenerative changes in kidney tissue (curative lower dose)**

*Effect on antioxidant levels*

In cisplatin control animals, the activities of antioxidant enzymes SOD, CAT, and GSH levels were significantly decreased with marked increase in LPO levels as compared with normal. Treatment with EECU significantly elevated the decreased activities of SOD, CAT, and GSH and inhibited the elevation in LPO in the dose-dependent manner (Table 2).

**Histological studies**

Treatment with cisplatin caused a marked necrosis in proximal tubules, degeneration of the tubular epithelial cells, and glomeruli. The treatment with EECU in both curative and prophylactic regimens caused regenerative changes and reduced the renal damage (Figs. 1-8).

**DISCUSSION**

The nephrotoxicity is the major side effect of cisplatin-based chemotherapy, as kidney accumulates it to a greater degree than other organs and is the major route for its excretion [17]. The cisplatin concentration in proximal tubular epithelial cells is about 5 times more than the serum concentration [18]. The nephrotoxic effect of cisplatin is

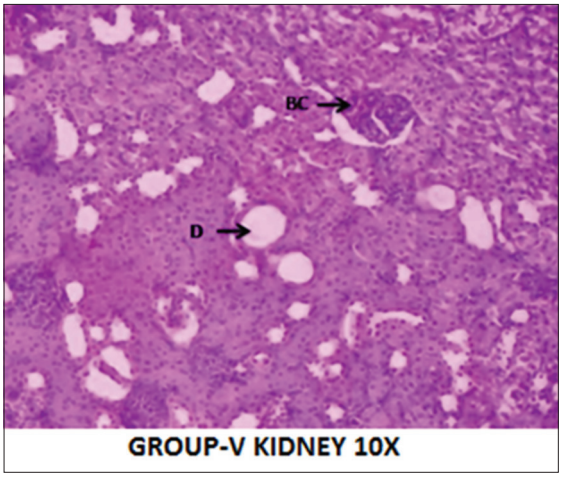


**Fig. 5: Section of rat kidney showing marked regenerative changes in renal tubule and Bowman's capsule (curative higher dose)**

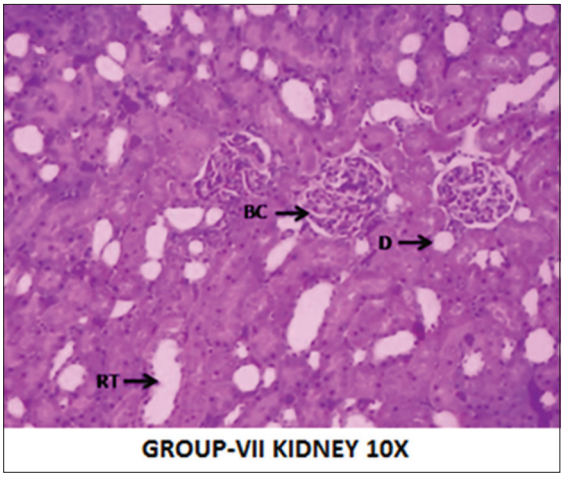


**Fig. 7: Section of rat kidney showing regenerative changes in kidney tissue and similar to normal cytoarchitecture (prophylactic higher dose)**

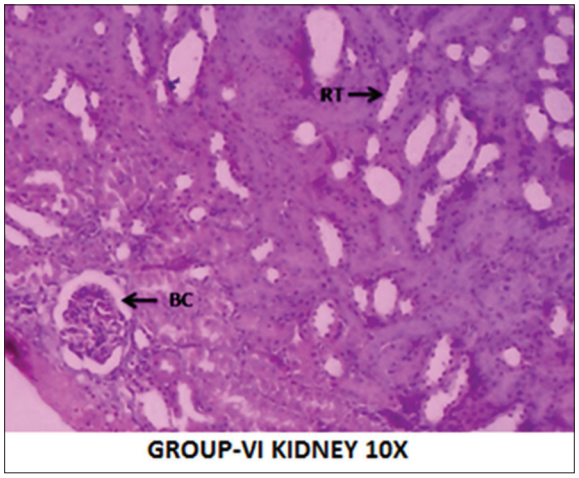


**Fig. 6: Section of rat kidney showing mild regeneration of kidney tissue (prophylactic lower dose)**

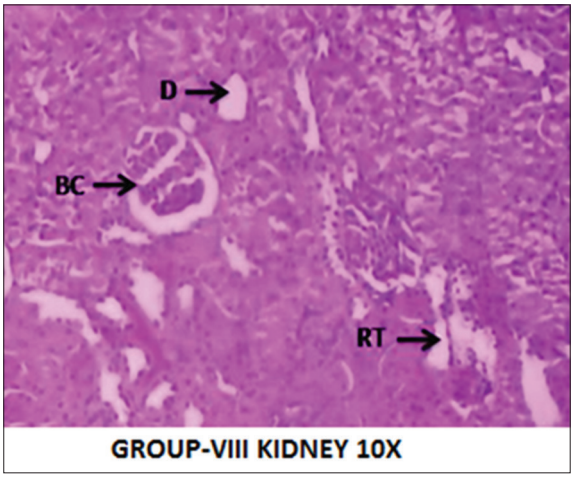


**Fig. 8: Section of rat kidney showing almost normal organization of Bowman's capsule and distal convoluted tubule (Only higher dose of ethanol extract of roots of *Catunaregam uliginosa*) RT: Renal tubule BC: Bowman's capsule**

dose limiting [19,8]. In the present study, cisplatin at a dose of 5 mg/kg bd. wt. induced nephrotoxicity in albino rats which was characterized by signs of injury, such as increased creatinine and BUN levels in plasma, raise in urinary total protein ($U_{TP}$), and reduced Clcr and results are in accordance with previous findings [20-22]. Increased SC and BUN levels observed in cisplatin-treated rats may be due to a reduction in glomerular filtration rate. The nephrotoxicity is a rapid process due to the reaction with the proteins in renal tubules. The renal damage is produced within 1 h after administration [23]. Hence, the presence of the protective agent in the renal tissues may reduce the toxic effects of cisplatin. This is the rationale behind the prophylactic regimen. Like *Hygrophila spinosa, Berberis aristata,* and *Salvia officinalis* EECU (200 and 400 mg/kg bd. wt.) have resulted in significant reversal of nephrotoxic effects induced by cisplatin in the dose-dependent manner in both curative and prophylactic regimens [24]. Oxidative stress is considered to play an important role in cisplatin-induced nephrotoxicity. MDA is considered to be one of the end products of LPO and an indicator of reactive oxygen species (ROS) production. Our results showed that cisplatin treatment-induced renal LPO, which was paralleled by the deterioration of renal structure and function. Earlier experimental findings also suggested that the free radicals and ROS are involved cisplatin-induced renal damage also due to the depletion of GSH concentration and antioxidant enzyme activities in the kidneys [25,26]. This study also demonstrated the reduced renal SOD, CAT, and GSH levels in the cisplatin-treated animals compared to the normal animals. These observations which support the mechanism of nephrotoxicity induced by cisplatin in animals are partially related to the depletion of the renal antioxidant system [27]. The stabilization of these renal parameters by the EECU was a clear indication of the improvement of the functional status of the kidney. The enhanced antioxidant activity of extract might be involved in the scavenging of free radicals generated from cisplatin. Moreover, renal histological examination revealed that cisplatin-induced renal damage which was indicated by the presence of degenerative tubules and degenerative glomeruli, whereas regenerative changes were observed in the group treated with ethanolic extract.

## CONCLUSION

Thus, the findings of the present study reveal that the EECU was efficient in the alleviation of cisplatin-induced nephrotoxicity in albino rats. Further, this study validates the ethnomedicinal use of this plant for renal disorders.

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