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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.830.839

Research Article
Nephroprotective Effect of Costus (*Saussurea costus*) Ethanolic Extract on Oxaliplatin®-induced Nephrotoxicity in Adult Male Wistar Rats

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Abstract

Background and Objective: Oxaliplatin® is an antineoplastic platinum-based compound; nephrotoxicity is one of its most serious side effects. This study aimed to explore the nephroprotective potential of Costus Ethanolic Extract (CEE) against Oxaliplatin®-induced nephrotoxicity. **Materials and Methods:** Adult male Wistar rats, weighting 140-160 g, were randomly divided into four groups: (1) Normal rats, (2) Rats ingested with CEE (67.08 mg kg^{-1} day^{-1}), (3) Rats injected (ip) with Oxaliplatin® (10 mg kg^{-1} $week^{-1}$) and (4) rats treated with CEE in combination Oxaliplatin® injection. **Results:** After six weeks of treatments, the results revealed that CEE ingestion along with Oxaliplatin® injection markedly minimized the Oxaliplatin®-induced renal deterioration; this was evidenced by the significant reduction in serum urea, creatinine, uric acid, Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1Beta (IL^{-1}β) and Sodium ion (Na^{+}) levels as well as kidney Malondialdehyde (MDA), Nitric Oxide (NO) and DNA fragmentation values. Controversially, a marked rise in serum Calcium, Potassium Ion (K^{+}) and Cluster of Differentiation 4 (CD4) levels besides renal Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) values. Similarly, the histopathological findings confirmed the biochemical ones as the CEE restored the Oxaliplatin®-induced histological degenerations. **Conclusion:** In conclusion, CEE exhibited nephron-protection efficiency against Oxaliplatin®-induced nephrotoxicity; this promising effect may be achieved through the antioxidant and radical scavenging activities of its constituents.

Key words: Nephrotoxicity, Oxaliplatin®, costus, antioxidant, histology, nephroprotective, serum calcium, acute kidney injury

Citation: Ashry, M., D.G. El-Sahra, D.A. Gaber, M.A. Mustafa and K.G. Abdel-Wahhab, 2021. Nephroprotective effect of costus (*Saussurea costus*) ethanolic extract on Oxaliplatin®-induced nephrotoxicity in adult male wistar rats. Pak. J. Biol. Sci., 24: 830-839.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several medications, such as non-steroidal anti-inflammatory drugs, antibiotics, antineoplastic medications and angiotensin-converting-enzyme inhibitors, may cause acute kidney injury[1]. Extreme, long and frequent episodes of acute kidney injury increase the risk that chronic kidney disease can progress. Acute and chronic renal failure are global public health issues with different features that must be considered in different areas worldwide; renal disorders affecting most organ systems can be managed and avoided using different therapeutic techniques[2].

Oxaliplatin® is one of the platinum agents of the third generation, developed after cisplatin and carboplatin. Recently, Oxaliplatin® has been used to treat bowel, pancreatic and stomach cancers[3]. Although the most common side effect is peripheral neuropathy[4]; with increased use of Oxaliplatin®, an increased number of acute kidney injury cases have been reported. In most cases, acute tubular necrosis was confirmed by renal biopsy[5]; only one case was reported as acute interstitial nephritis histopathologically[6].

Several mechanisms, including the generation of Reactive Oxygen Species (ROS), are thought to mediate the nephrotoxicity of the platinum anticancer drugs[7]. A determinant stage that leads to oxidative stress and acute renal failure is the depletion of platinum drug-induced Glutathione (GSH). Kanou *et al.*[8] have demonstrated similar *in vitro* nephrotoxicity in the two platinum drugs (Cisplatin® and Oxaliplatin®). Oxaliplatin® is the main active platinum complex found in plasma ultrafiltrate of treated patients and Oxaliplatin® plasma biotransformation products are unlikely to contribute to its effectiveness or toxicity[9]. The mechanism of platinum compounds nephrotoxicity is not fully understood[10]. In a rat model of Oxaliplatin-induced neuropathy, oxidative stress was previously identified as a main biomolecular dysfunction showing a relationship between oxidative damage of the nervous system and pain[11].

Several years ago, herbal medicines were used worldwide for the treatment of different diseases due to the presence of beneficial chemical elements in them. In essence, plants' medicinal potential lies in their phytochemical constituents that, when applied to the human body, produce a definite pharmacological action. Phytochemicals are naturally occurring in medicinal plants' leaves, vegetables and fruits that have their defence mechanisms and protection ions from various diseases[12].

Costus *(Saussurea costus)* is commonly found in most forest areas, including Cameroun, Nigeria, Guinea, Ghana, South Africa, Senegal and most tropical African regions, mostly in higher rainfall areas[13,14]. Costus is commonly used in tropical Africa as a medicinal plant and pharmacological studies have confirmed many uses and it has some value as an ornamental plant[14]. The plant is used as a remedy for cough, inflammation, laxative, purgative, diuretic and treatment for many other diseases[15]. Furthermore, ethanol extracts from the root of costus have been reported to have nephroprotective activities[16]. *Saussurea costus* has been screened for numerous pharmacological activities including antioxidant[17], antihepatotoxic[18], anticancerous, anti-inflammatory[19], anti-ulcerous[20], antimicrobial[21], immunomodulatory[22], cardioprotective[23] antiepileptics[24] and antihyperlipidemic activities[25]. These valuable activities may be the result of the presence of bioactive molecules such as costunolide, sesquiterpenoids, dehydrocostus lactone, monoterpenes, cynaropicrin, flavonoids, lignans, triterpenes, steroids and glycosides[16].

Therefore; the current study was performed to investigate the potential modulatory effects of costus roots ethanolic extract against kidney injury and fragmentation of DNA caused by Oxaliplatin®.

MATERIALS AND METHODS

Study area: This study was carried out during the period from September-December, 2019 at the laboratories of the Department of Medical Physiology, National Research Centre, Egypt; Department of Zoology, Faculty of Science, Al-Azhar University and Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Helwan University, Egypt.

Plant and extraction: *Costus* (*Saussurea costus*) roots were obtained from Imtinan Company, Egypt; then identified and authenticated by scientific botanists and found carrying a taxonomic serial number 780691. The ethanolic extraction process of the dry powdered roots was carried out according to the modified method of Filipiak-Szok *et al.*[26]; the powder was soaked absolute ethanol (10% w/v) at room temperature for 24 hrs under continuous stirring; then the mixture was filtered through sterile filter paper (Whatman number 42, England). The solvent was evaporated using a rotary evaporator and then the extract was stored at -20°C until further use.

Determination of total extract yield: The combined extracts were transferred to a quick fit round bottom flask with known weight (W_1), freeze-dried and weighed again (W_2) and finally the yield calculated from the following formula:

$$\text{Extract yield (g g}^{-1}\text{ crude herb)} = \frac{W_2\text{-}W_1}{W_3}$$

where, W_1 is the weight of clear and dry quick fit flask in grams, W_2 is the weight of the flask after lyophilization in grams and W_3 is the weight of the crude powdered herb in grams.

Determination of total phenolic content: Phenolic content of the CEE was performed by dissolving 5 mg of the extract in a 10 mL mixture of acetone and water (6:4 v/v). Then, a sample of 0.2 mL was mixed with 1.0 mL of Folin-Ciocalteu reagent (10 fold diluted) and 0.8 mL of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using Cary 100 UV-Vis spectrophotometer, Agilent Technologies, Santa Clara, United States. Estimation of phenolic compounds as catechin equivalents was carried out using a standard curve[27].

DPPH radical scavenging activity: The capacity of antioxidants of CEE to quench DPPH radical was determined as previously described[28]. In this method, a certain amount of the crude extract was dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 mL of this solution was completed to 4 mL by methanol and 1 mL DPPH solution (6.09×10^{-5} mol L^{-1}), in the same solvent, was then added. The absorbance of the mixture was measured at 516 nm after 10 min standing. The reference sample (blank) was 1 mL of DPPH solution and 4 mL methanol. Triplicate measurements were made and the percentage of radical scavenging activity was calculated according to the equation below as described by Nogala-Kalucka *et al.*[28].

$$\text{RSA (\%)} = \left(\frac{A_{\text{control sample}} - A_{\text{sample extract}}}{A_{\text{control sample}}}\right) \text{x } 100$$

Estimation of reducing power: The reducing power of the used extract was determined according to the method described by Sethiya *et al.*[29] with some modifications. From both extract and ascorbic acid, 0.5 mL of different concentrations (50, 100, 200, 400 and 800 µg mL^{-1}) was mixed with 2.5 mL phosphate buffer (pH 7.4) and 2.5 mL potassium ferricyanide (0.1 M); the mixture was kept at 50°C in a water bath for 20 min; then after cooling, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. Immediately, 2.5 mL of the upper layer of the solution was mixed with 2.5 mL distilled water and 0.5 mL of a freshly prepared ferric chloride solution (40% w/v). The absorbance of the sample and standard was measured at 700 nm. The reducing power was calculated as equivalent to ascorbic acid from the reducing power standard curve of ascorbic acid. Control blank included mixture components without sample or standard.

Animals and experimental design: Adult male albino rats, weighting 140-160 g, were obtained from the Animal Colony, National Research Centre, Egypt; the animals were maintained under temperature (25±1°C) and light-controlled conditions (12/12hrs light/dark cycle) on free access to food and water for a week before starting the experiment for acclimatization. The animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the procedures approved by the Ethics Committee of the National Research Centre (FWA 00014747) which follows the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved the study proposal. After being acclimatized with experimental room conditions, the animals were divided randomly into four groups (10 animals each) as group (1) included healthy control rats orally received water (0.5 mL day^{-1}) for consecutive six weeks, group (2) included healthy rats ingested with CEE (67.08 mg kg^{-1}/day) for a similar period, group (3) included healthy rats intraperitoneally intoxicated with Oxaliplatin® (10 mg kg^{-1} once a week) for six weeks and group (4) rats administrated with CEE (same dose) combined with Oxaliplatin® injection (same dose) for consecutive six weeks.

Blood and tissue sampling: At the end of the experimental period, rats were weighed then fasted overnight; then following anaesthesia (inhalation with diethyl ether), blood specimens were withdrawn, left to clot and cool-centrifuged at 3000 rpm for 10 min; the sera were separated, divided into aliquots and stored at -80°C till biochemical measurements were carried out as soon as possible. After blood collection, all animals were sacrificed soon by decapitation and then both kidneys were dissected out; the left kidney was washed in saline, dried, rolled in a piece of aluminium foil and stored at -80°C for determination of oxidative stress markers and DNA fragmentation, while the right kidney was preserved in formalin-saline (10%) buffer for histopathological processing and microscopic examination.

Biochemical determinations: Serum biochemical measurements were carried out spectrophotometrically; serum urea, creatinine, uric acid, albumin, Calcium, Sodium and Potassium levels were determined using reagent kits purchased from DiaSys Diagnostic System GmbH, Germany.

Inflammatory cytokines: Using the ELISA technique (Dynatech Microplate Reader Model MR 5000, Canada), serum Tumour Necrosis Factor Alpha (TNF-α), Interleukin-1 Beta (IL-1β) and CD4 concentrations were measured using reagent kits purchased from SinoGeneClon Biotech Co., Ltd, No. 9 BoYuan Road, YuHang District 311112, Hang Zhou, China.

Tissue homogenization and estimation of oxidative stress markers: A specimen of each kidney was subjected to ultrasound homogenization in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); then the homogenate was centrifuged at 5000 rpm for 20 min to remove the nuclear and mitochondrial fractions and finally, the clear supernatant was divided into aliquots and stored at -80°C till estimation of oxidative stress markers. Reduced Glutathione (GSH) and Nitric Oxide (NO) levels, as well as Superoxide Dismutase (SOD) and Catalase (CAT) activities, were estimated using reagent kits obtained from Biodiagnostic, Dokki, Giza, Egypt. However, the level of Malondialdehyde (MDA), the most abundant individual lipid peroxidation end product resulting from a breakdown in biological systems and used as an indirect index for lipid peroxidation, was determined chemically as described by Gomaa *et al.*[30] through MDA reaction with Thiobarbituric Acid (TBA) forming a pink complex that can be measured photometrically.

DNA fragmentation: The percentage of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay according to the quantitative method used for grading the DNA damage[31]. The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet and the proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the equation below which was described by Ashry *et al.*[31]:

$$\text{DNA fragmentation \%} = \frac{\text{A supernatant}}{\text{A supernatant} + \text{A pellet}}$$

Histopathology: Paraffin sections, 5 µm thick, were stained with Abdel-Wahhab *et al.*[32] and investigated by light microscope.

Statistical analysis: Multiple comparisons among means were carried out using one-way analysis of variance (ANOVA) followed by post hock (Tukey) test at $p \geq 0.05$ according to Ashry *et al.*[33]; this analysis was carried out using Statistical Analysis System (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

RESULTS

The *in vitro* results revealed that the powdered costus gives a considerable yield amount that was close to 15.9% post ethanolic extraction. Moreover, Costus Ethanolic Extract (CEE) has a high content of Total Phenolic Compounds (TPC) which was about 22.6% of the extract, performed extreme Radical Scavenging Activity (RSA) that didn't less than 95.2% and valuable reducing power that gave absorbance 0.5, 0.7, 0.9, 1.1 and 1.3 at 700 nm for its gradient concentrations 50, 100, 200, 400 and 800 µg mL^{-1}, respectively (Fig. 1-2).

The present study showed significant increases in serum TNF-α and IL1β levels and kidney DNA fragmentation percentage coupled with significant a drop in serum CD4 post-Oxaliplatin®intoxication compare to the corresponding

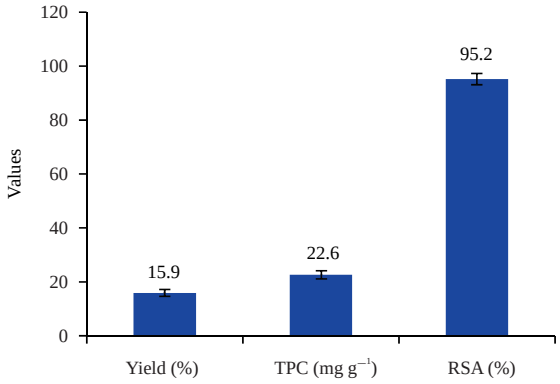

Fig. 1: Mean values of three replicates of the yield, total phenolic content (TPC) and radical scavenging activity (RSA) of the CEE

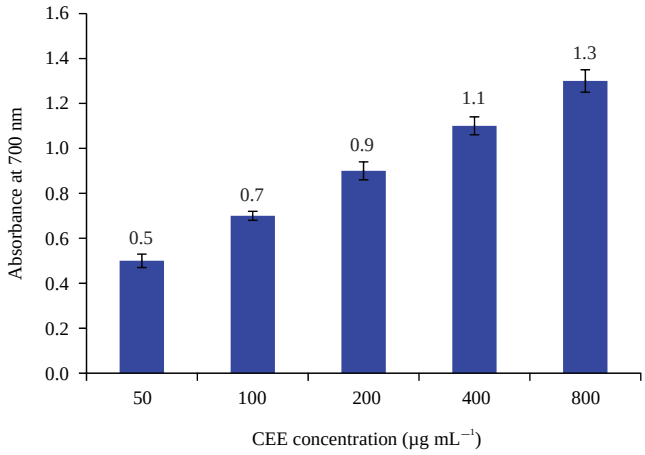

Fig. 2: Mean values of three replicates of the reducing power of *Costus* (*Saussurea costus*) ethanolic extract

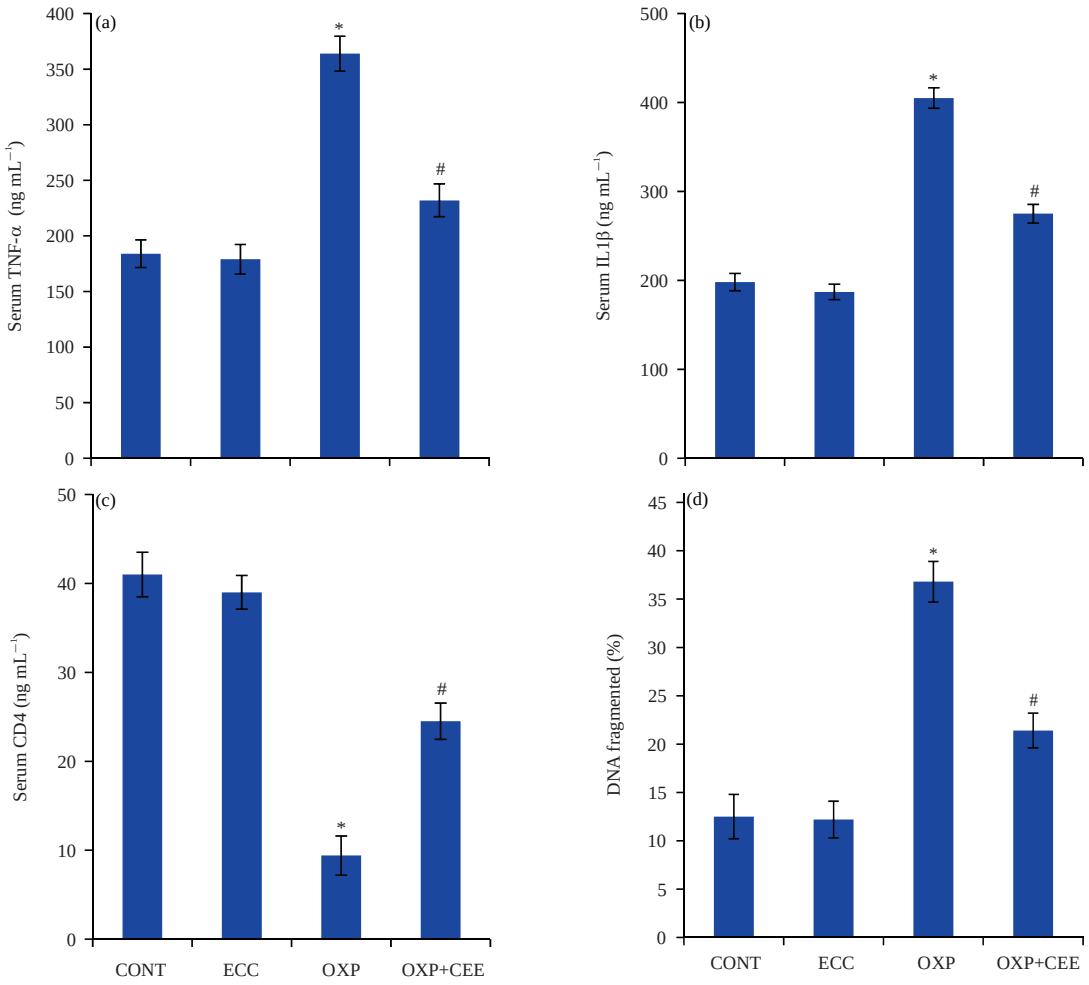

Fig. 3(a-d): (a) Serum TNF-α, (b) IL1β, (c) CD4 and (d) DNA fragmentation of control, Oxaliplatin® (OXP)-intoxicated and CEE-treated male albino rats

*Significantly different from the control group, #Significantly different calculated from the OXP group ($p \leq 0.05$)

Table 1: Serum renal function markers (urea, creatinine, uric acid, natrium, potassium and calcium) of control, oxaliplatin®-intoxicated and CEE-treated male albino rats

Parameters	Control	CEE	OXP	OXP with CEE
Urea (mg dL^{-1})	51.00±8.1	52.00±10.1	99.00±6.7*	67.00±5.9#
Creatinine (mg dL^{-1})	1.23±0.25	1.26±0.29	2.30±1.2*	1.70±0.24#
Uric acid (mg dL^{-1})	4.30±1.5	4.11±2.1	7.51± 3.5*	5.90±1.9#
Potassium (mmol L^{-1})	8.40±0.6	8.20±0.4	10.22±0.7*	8.66±0.5#
Sodium (mmol L^{-1})	138.00±11.9	142.00±12.6	120.00±18.1*	132.00±9.5#
Albumin (g dL^{-1})	5.10±0.2	5.20±0.8	2.60±0.3*	4.30±0.17#
Total calcium (mg dL^{-1})	9.30±1.6	9.80±0.75	6.20±1.2*	8.70±2.1#

Data are expressed as Mean±standard error. *Significantly different from the control group, #Significantly different from the OXP group at $p \leq 0.05$ using one way ANOVA. OXP: Oxaliplatin®, CEE: Costus ethanolic extract

values of healthy control. Interestingly, co-administration of CEE besides Oxaliplatin® injection succeeded to inhibit the Oxaliplatin®-induced toxicity as it significantly decreased TNF-α and IL1β serum levels and DNA fragmentation percentage and significantly increased the serum CD4 level close to the values of healthy control (Fig. 3a-d).

The data of Table 1 shows that Oxaliplatin® (OXP) injection led to a significant elevation in the levels of serum urea, creatinine, uric acid and Potassium matched with a significant reduction in serum Natrium, albumin and total calcium levels in comparison to values of the control group. Favourably, CEE administration in line with OXP injection markedly ameliorated the above mentioned renal function markers.

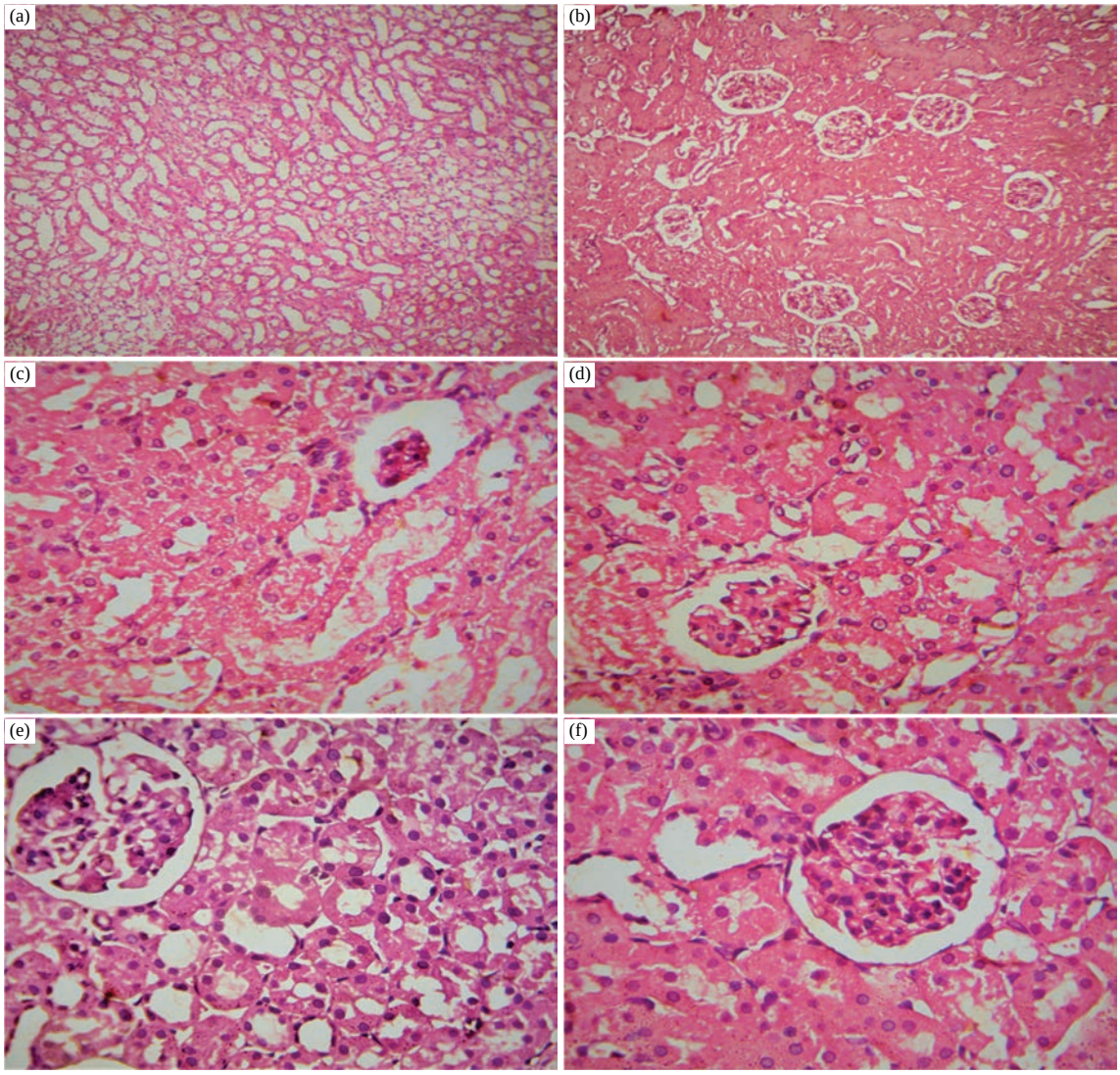

Fig. 4(a-f): Photomicrographs of kidney sections

(a) Control group showing the normal histological structure of the tubules in the medullary portion, (b) CEE treated group showing the normal histological structure of glomeruli and tubules in the cortical portion, (c) Oxaliplatin® chemotherapy-treated group showing Atrophied Glomerulus (AG) and degenerated renal tubules, (d) Oxaliplatin® chemotherapy-treated group showing fragmented glomeruli (arrow) and degenerated renal tubules, (e) Group treated with CEE with Oxaliplatin® chemotherapy showing improvement in tubules and glomeruli (G) structure and (f) Group treated with CEE with Oxaliplatin® chemotherapy showing Minimal histological changes (H and E×400)

Regarding renal oxidative stress markers (Table 2), the obtained results displayed that the OXP-induced nephrotoxicity performed a significant increase in renal Malondialdehyde and NO levels coupled with a marked drop in renal GSH level and renal SOD and CAT activity. Controversially, the animals that given CEE and OXP together showed a significant decrease in kidney MDA and NO levels coupled with a significant increase in renal GSH, SOD and CAT values in comparison to the data of the OXP group.

Results of histopathological examination of kidney sections are illustrated and described in Fig. 4a-f. Control group: Photomicrograph of kidney section from the control group showing the normal histological structure of the tubules in the medullary portion; negative control group: Photomicrograph of kidney section from CEE treated group showing the normal histological structure of Glomeruli (G) and tubules in the cortical portion; Oxaliplatin® group: Photomicrograph of Kidney section from Oxaliplatin® chemotherapy-treated group showing Atrophied

Table 2: Renal oxidative stress markers (MDA, NO, GSH, SOD and CAT) of control, oxaliplatin®-intoxicated and CEE-treated male albino rats

Parameters	Control	CEE	OXP	OXP with CEE
MDA (μmol g^{-1} tissue)	168.4±10.4	152.4±9.45	405.40±18.5*	245.50±15.1#
NO (μmol g^{-1} tissue)	3.4±0.27	3.2±0.14	9.46±0.22*	4.90±0.14#
GSH (nmol g^{-1} tissue)	441.6±20	495.4±31	210.00±19*	375.00±21#
SOD (U g^{-1} tissue)	122.4±15.4	133.4±17.4	41.40±12.4*	84.50±11.4#
CAT (U g^{-1} tissue)	11.4±2.4	12.1±1.9	4.51±2.4*	8.92±1.4#

Data are expressed as Mean±standard error. *Significantly different from the control group, #Significantly different from the OXP group at $p \leq 0.05$ using one way ANOVA followed by *post hoc* (Tukey) test, OXP: Oxaliplatin®, CEE: Costus ethanolic extract, MDA: Malondialdehyde, NO: Nitric oxide, GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase

Glomerulus (AG) and degenerated renal tubules and Oxaliplatin® with CEE group: Photomicrograph of kidney section from the group treated with CEE with Oxaliplatin® chemotherapy showing improvement in tubules and glomeruli (G) structure.

DISCUSSION

The present work aimed to evaluate the possible protective effects of ethanolic extract of *Saussurea costus* against kidney toxicity and DNA damage resulting from Oxaliplatin® in male Wistar rats. The kidney is a vital organ to fulfil many important functions including homeostasis maintenance and control of the extracellular environment such as detoxification and excreting toxic metabolites and drugs[34]. Oxaliplatin® is one of the anti-cancer drugs that have been used to treat bowel, pancreatic and stomach cancers[3]; it leads to many systemic complications that limit its dose and duration use, including acute kidney injury.

Besides, the results also showed that Oxaliplatin® caused a disturbance in the oxidative stress markers as evidenced by the significant decrease in the activity of antioxidant enzymes (CAT and SOD), significant depletion of GSH and elevated MDA production in renal tissue. These findings are in agreement with those of Yamada *et al*.[3] and Biswas *et al*.[35].

Nephrotoxicity induced by Oxaliplatin® through apoptosis and necrosis, vascular factors and tubules inflammation[36]. The development of injury to the renal tubules occurs due to Oxaliplatin®-induced oxidative stress[37]. The Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) production[4] alter the structure and function of cellular membranes[38]. In addition to their accumulation in the kidney and lysosomes[39] explained the mechanisms for OXP-induced acute nephropathy[4].

While numerous OXP-induced nephrotoxicity mechanisms have been identified, such as mitochondrial dysfunction, inflammation, DNA damage, oxidative stress and apoptosis, the exact mechanism is not well understood[40]. Therefore, Oxaliplatin®-induced nephrotoxicity can be prevented by free radical scavengers and antioxidant agents. The research results showed that Oxaliplatin® injection produced pathophysiological nephrotoxicity which was achieved from the significant increase in serum creatinine, urea, uric acid and potassium levels accompanied by a marked decrease in the level of serum albumin total calcium and natrium. Increased levels of urea and creatinine run in line with that of Waly *et al*.[41] who attributed that to the decreased glomerular filtration rate.

Administration of CEE along Oxaliplatin® intoxication succeeded to modify and regenerate kidney cell damage and normalization of serum levels of urea, creatinine, uric acid and electrolytes that were altered by Oxaliplatin® intoxication. It also decreased damage to the renal DNA caused by Oxaliplatin®. CEE was found rich in bioactive molecules, such as dehydrocostus lactone, costunolide, cynaropicrin, monoterpenes, sesquiterpenoids, flavonoids, lignans, triterpenes, steroids and glycosides[27] as a high total phenolic content was found in CEE. Its therapeutic activities, including the ability to reduce oxidative stress, are very familiar with these compounds[42]. The reason for the inhibition of Oxaliplatin®-induced alterations could be the reduction of oxidative stress; CEE could be a cytoprotective agent as it showed high free radical scavenging activity (*in vitro* results) that causes extensive damage to cell component[43].

Study observations showed that the prevention of lipid peroxidation associated with restoration of the depleted renal GSH and reactivation of renal antioxidant enzymes (SOD and CAT) was prevented by CEE treatment, indicating that the plant has significant anti-peroxidation and anti-oxidant activities. Administration of CEE with Oxaliplatin® resulted in a significant increase in antioxidant activities in renal tissue compared to the Oxaliplatin® group. These results are in agreement with the study of Ezejiofor *et al*.[44]; Tousson *et al*.[36] and Umoh *et al*.[45] who reported that CEE may serve as a potential source of biopharmaceutical agents with the anti-oxidative property as it was able to restore the antioxidative stress markers close levels of normal control.

Besides, the study showed that Oxaliplatin® injection resulted in a significant decrease in serum CD4 level compared to that of normal controls; this result agrees with a previous

study[46]. Helper CD4+ T cells play a crucial role in adaptive immunity by conditioning the environment and, essentially, modulating the activity of other immune cells through the production of cytokines that can perform cross-presentation to CD8+ T cells[47]. There are limited studies regarding the effects of Oxaliplatin® and the predecessor Platinum-based agents on CD4+ T cells. In the present study, we noted that the levels of the pro-inflammatory cytokines TNF-α and IL-1β were increased markedly after OXP chemotherapy in rats. These findings are similar to those observed in other previous studies[48, 49]. Stimulated inflammatory cytokines, such as TNF-α or IL-1β, serve as a key regulator of apoptosis in cells[50]. After Oxaliplatin® injury, the expression of the number of inflammatory cytokines and chemokines is increased in the kidney[51]. In the present study, OXP increased both TNF-α and IL-1β expression levels. It was stated that Oxaliplatin®-nephrotoxicity resulted in up-regulation in TNF-α, IL-1β, Macrophage Inflammatory Protein-2 (MIP-2), Monocyte Chemoattractant Protein-1 (MCP-1), Intercellular Adhesion Molecule 1 (ICAM-1) and Transforming Growth Factor-Beta (TGF-β)[52]. The anti-inflammatory effects shown in the present study indicate that the CEE may have immunomodulatory properties. The phytochemical analysis of the crude CEE has shown that its main chemical components are phenolics and flavonoids, which have antioxidant[53].

After Oxaliplatin® therapy, histopathological changes were noticed in the kidney tissues in form of acute tubular necrosis, confirming irreversible kidney injury; besides, extreme atrophy of glomerulus was also found and apparent due to its reduced size. Moreover, marked dilation of proximal convoluted tubules with slogging of almost the entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstitium is also an indication of Oxaliplatin®-induced renal necrosis. The changes in the present study are parallel to the report reported by Yamada *et al.*[3].

The phytochemical analysis found in costus showed a high concentration of alkaloids and flavonoids that may be responsible for their anti-inflammatory activities[54]. Regarding the CEE-Oxaliplatin® treated group, the histological features were consistent with previous studies on the ethanol extracts of costus that have been concluded to have a protective and anti-inflammatory effect on the kidney[55]. Also, the present study showed that CEE administration seems to improve the nephrotoxicity or pathological kidney conditions, as was monitored from the significant regeneration of OXP destroyed glomerulus and Bowman's capsule.

CONCLUSION

This study demonstrated that *Saussurea costus* ethanolic extract can protect against nephrotoxic effects of Oxaliplatin®; this effect evidenced by the up-regulation of antioxidant battery and suppression of oxidative markers under the antioxidant properties of the phytochemical constituents of CEE. As a supplement, costus has a promising pharmaceutical efficacy for managing Oxaliplatin®-resultant renal damage.

SIGNIFICANCE STATEMENT

This study discovered the protective effect of ethanolic extract of *Saussurea costus* against nephrotoxicity; so *Saussurea costus* may be a beneficial dietary supplement in case of treatment with Oxaliplatin®. This study may help the researchers to uncover the critical areas of using an extract of *Saussurea costus* that many researchers were not able to explore.

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