Ameliorative effect of costus ethanolic extract against Oxaliplatin-induced hepatotoxicity in adult rats

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Background and objective
Cancer is a disease associated with an abnormal proliferation and growth of living cells; treatment with the anticancer therapy, Oxaliplatin (OXP) results in hepatotoxicity. The objective of this study was to evaluate the protective effect of costus ethanolic extract (CEE) against OXP-induced hepatotoxicity in a trail to improve its clinical use.
Materials and methods
Adult male Wistar rats (150–180 g body weight) were randomly divided into four groups (10 rats each): (a) healthy control group, (b) healthy rats treated orally with CEE (50 mg/kg/day), (c) rats injected intraperitoneally with OXP (10 mg/kg once/week), and (d) rats treated with CEE in combination with OXP.
Results and conclusion
After 6 weeks of treatment, the results revealed that CEE succeeded to decline OXP-induced hepatotoxicity; this was evidenced by the significant reduction in serum alanine aminotransferase (ALAT), aspartate aminotransferases (ASAT), GGT, alkaline phosphatase (ALP), total cholesterol, triglycerides, low dense lipoprotein-cholesterol (LDL-c), tumor necrosis factor-alpha (TNF-α), Interleukin -1 Beta (IL-1β), and alpha-fetoprotein values as well as hepatic malondialdehyde, nitric oxide, and DNA fragmentation coupled with a marked rise in serum CD4, albumin and high dense lipoprotein-cholesterol (HDL-c) levels, and hepatic glutathione, superoxide dismutase, and catalase values. These effects agonized the structural restoration of the histological picture of liver. It could be concluded that CEE succeeded to a great extent to counteract the oxidative stress of OXP and protect the liver against its toxic effects; CEE may be considered as a promising supplement-candidate for the protection of liver against the side effects of that anticancer drugs.

Keywords:
costus, hepatotoxicity, immunomodulation, Oxaliplatin, rat

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Introduction

Drug-induced hepatotoxicity is one of the major concerns in medical practice. Although it is relatively uncommon, drug-induced liver injury is the leading cause of acute liver failure in the world and a major reason for liver transplantation [1].

Oxaliplatin (OXP), a third-generation platinum chemotherapeutic agent, is widely used in the treatment of several cancers such as colorectal cancer and gastric cancer [2]. OXP-based chemotherapy for colorectal liver metastases has increased resection rates and improved outcomes, and is therefore recommended as the first-line basic chemotherapeutic drug [2,3]. However, OXP-induced liver injury is a primary limiting factor of OXP-based chemotherapy in patients with colorectal liver metastases [4]. Studies have revealed OXP-induced liver injury in patients who underwent preoperative OXP-based chemotherapy, with an incidence rate of 19–78% [5]. Other reports have shown that OXP-induced sinusoidal injury, one of the distinct drug-specific side effects of OXP, is associated with intraoperative bleeding and postoperative morbidity, and early recurrence and decreased overall survival [6]. The pathological features of OXP-induced liver injury include hepatic sinusoidal dilatation, intrahepatic sinus platelet aggregation, hepatic steatosis, and clinically important adverse effects characterized by a bluish hue in the liver, splenomegaly, and thrombocytopenia [7]. To overcome these side effects, an effective adjuvant drug that protects the liver against damage caused by OXP is imperative.

At present, very little is known about the pathophysiological mechanisms that underlie OXP-

induced liver injury. OXP has been confirmed to cause liver oxidative stress response through certain known mechanisms. Robinson *et al.* [8] reported that oxidative stress-related genes (Mt1, HO1, and SOd3) were upregulated in the liver following OXP chemotherapy, indicating that oxidative stress plays an important role in OXP-induced liver injury. By generating reactive oxygen species (ROS), OXP causes a series of reactions, such as oxidative injury of normal hepatocyte mitochondria, as well as injury, falloff, and local edema of sinusoidal endothelial cells, thereby causing chemotherapy-related liver injury [9].

Plants have a long-time history in medicine. For centuries, many people have developed different herbal medicines using locally available plants as a remedy to their numerous health challenges. When these medicinal plants are excessively consumed, they could result in the damage of some body tissues and their functions [10].

Costus (*Saussurea costus*) commonly known as 'Kuth' from the family *Asteraceae* is an important medicinal plant. Its roots are widely used in folk medicine. Several studies have reported that the root of costus exhibited antimicrobial and anti-nematode activity [11], hepatoprotective activity [12], antiulcer activity [13], and anti-inflammatory activity [14]. The authors reported the presence of caffeic acid derivatives, chlorogenic acid (1S-(1, 3, 4, 5)-3-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyloxy-1, 4, 5-trihydroxycyclohexanecarboxylic acid), in costus for the first time by high performance liquid chromatography (HPLC) [15]. Chlorogenic acid exhibited antioxidant activity [16]. Although the plant has been reported to contain caffeic acid derivatives (like syringic acid and chlorogenic acid), the antioxidant activity of the plant has now been studied for the first time using its ability to scavenge 1,1-diphenyl-2-picrylhydrazyl, nitric oxide (NO), superoxide radicals, along with its ability to inhibit lipid peroxidation and glutathione (GSH) oxidation. This study aimed to evaluate the ameliorative effect of costus ethanolic extract (CEE) against hepatotoxicity induced by OXP.

Materials and methods

Plant materials and extraction

Costus roots were obtained from Imtinan Company, Nasr City, Cairo, Egypt, which were identified and authenticated by scientific botanists. The plant was found carrying a taxonomic serial number 780691. The ethanolic extract of the dry powdered roots was carried out according to the modified method of Filipiak-Szok *et al.* [17]; 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of CEE was determined using the method previously described [18]. Reducing power of the extract was determined according to the method described by Sethiya *et al.* [19].

HPLC analysis of phenolic constituents

High performance liquid chromatography (HPLC) analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil C18 column (4.6 mm×250 mm id, 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8–12 min (60% A), 12–15 min (85% A), and 15–16 min (82% A). The multiwavelength detector was monitored at 280 nm. The injection volume was 10 μl for each of the sample solutions. The column temperature was maintained at 35°C.

Animals and experimental design

Forty adult male albino rats (150–180 g) were obtained from the Animal Colony, National Research Centre, Egypt; the animals were maintained under temperature-controlled (25±1°C) and light-controlled (12/12 h light/dark cycle) conditions with 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 institution's criteria according to the procedures approved by the Ethics Committee of the National Research Centre (FWA 00014747) that follows the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication No. 85-23, revised in 1985). After the animals were acclimatized to experimental room conditions, they were divided randomly into four groups (10 animals each) as follows: group 1 healthy control rats orally received 0.5 ml water for consecutive 6 weeks; group 2 healthy rats orally ingested with CEE (50 mg/kg/day) for six consecutive weeks, group 3 healthy rats intoxicated intraperitoneally with OXP (10 mg/kg/week) for 6 weeks, and group 4 rats intoxicated with OXP combined with ingestion of CEE for 6 weeks at the mentioned doses.

Blood and tissue sampling

At the end of the treatment period (6 weeks), rats were weighed and then fasted overnight. Following anesthesia (inhalation with diethyl ether), blood specimens were withdrawn from the retro-orbital

plexus using heparinized and sterile glass capillaries; whole blood specimens were cool centrifuged at 3000 rpm for 10 min and the sera were separated, divided into aliquots, and stored at −80°C till biochemical measurements, which were carried out immediately. Then after blood collection, the animals were killed soon, and then the liver of each animal was dissected out. One part of the liver of each animal was washed in saline, dried, rolled in a piece ofaluminum foil, and stored at −80°C for both biochemical determinations and DNA fragmentation. Another portion of the liver was soaked in formalin-saline (10%) buffer for histopathological processing and microscopic examination.

Biochemical determinations

Serum aspartate aminotransferases (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), and GGT activities were determined spectrophotometrically using reagent kits purchased from Human GesellSchaft fur Biochemical und Diagnostic mbH, Germany, while serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, albumin, and total protein levels were determined using reagent kits purchased from DiaSys Diagnostic Systems GmbH, Germany. Serum tumor necrosis factor-alpha (TNF-α), Interleukin -1 Beta (IL-1β), CD4, and αFP concentrations were measured using ELISA kits purchased from SinoGeneClon Biotech Co. Ltd, No.9 BoYuan Road,YuHang District 311112, Hang Zhou, China. Hepatic levels of GSH and NO and activities of superoxide dismutase (SOD) and catalase (CAT) were estimated using reagent kits obtained from Biodiagnostic, Giza, Egypt. However, malondialdehyde (MDA) level was determined chemically as described by Ruiz-Larnea *et al.* [20].

DNA fragmentation percentage

The percentage of DNA fragmentation was assayed according to the quantitative method used for grading the DNA damage [21].

Histopathology

Paraffin sections of 5 μm thickness were stained with hematoxylin and eosin [22] and investigated by light microscopy.

Statistical analysis

Comparisons between means were carried out using one-way analysis of variance, followed by post hock (Tukey) multiple comparisons test at *P* value less than or equal to 0.05 according to Steel and Torrie [23].

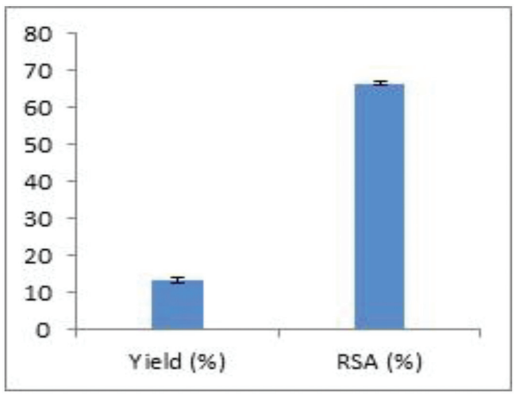
Results

The yield, radical scavenging activity, and reducing power of the CEE are shown in Figs 1 and 2. Mostly 16 phenolic compounds were identified in CEE using HPLC analysis. The compounds identified were found to include high contents of naringenin, chlorogenic acid, ferulic acid, taxifolin, gallic acid, and caffeic acid (Fig. 3 and Table 1).

In comparison to the control group, the obtained results showed a significant increase in TNF-α, IL-1β, and alpha-fetoprotein (AFP) level coupled with a significant decrease in CD4 post-OXP intoxication. Interestingly, administration of rats with CEE besides OXP intoxication led to a marked reduction in the measured inflammatory cytokines (TNF-α and IL-1β) and tumor marker (AFP) associated with a significant increase in serum CD4 level to values close to those of the normal control group when compared with OXP-intoxicated animals (Fig. 4).

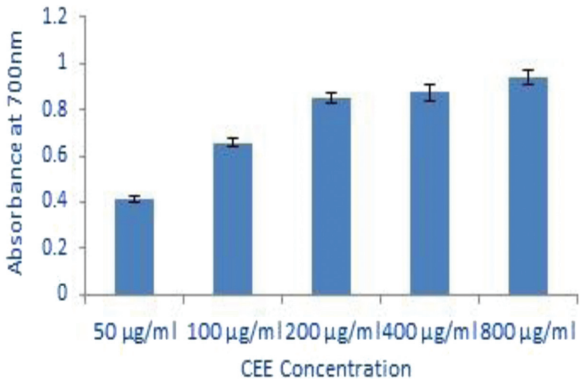
The data in Table 2 show that the administration of rats with CEE alone did not disturb the activity of

Figure 1



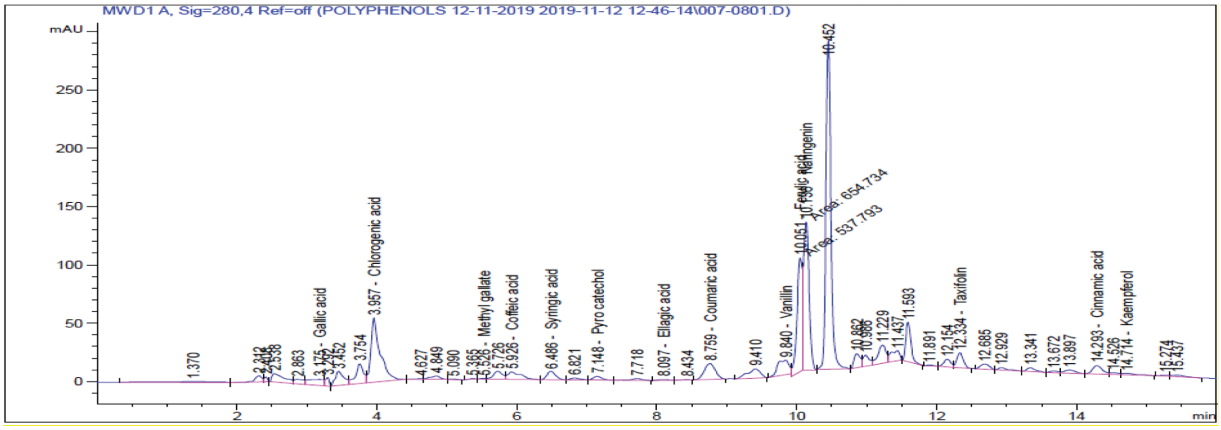
The yield (%) and radical scavenging activity (%) of three replicates of ethanolic extract of costus dry powdered roots.

Figure 2



Reducing power of three replicates of the ethanolic extract of costus dry powdered roots.

Figure 3



HPLC analysis of phenolic constituents of costus ethanolic extract.

Table 1 Phenolic constituents of ethanolic extract of costus using HPLC analysis

	Area	Concentration (µg/ml=µg/6.8 mg)	Concentration (µg/g)
Gallic acid	77.66	6.23	232.49
Chlorogenic acid	508.86	39.58	1477.00
Catechin	0.00	0.00	0.00
Methyl gallate	4.14	0.06	2.33
Coaffeic acid	87.66	3.17	118.27
Syringic acid	63.85	2.17	81.15
Pyro catechol	28.89	2.80	104.38
Rutin	0.00	0.00	0.00
Ellagic acid	6.58	0.38	14.08
Coumaric acid	150.73	2.43	90.79
Vanillin	143.40	2.24	83.58
Ferulic acid	537.79	16.74	624.59
Naringenin	654.73	40.05	1494.47
Taxifolin	82.11	9.36	349.28
Cinnamic acid	70.85	0.75	27.91
Kaempferol	20.59	1.68	62.50

HPLC, high performance liquid chromatography.

serum ASAT, ALAT, ALP and GGT, while OXP injection led to a significant elevation in the activity of these parameters when both groups were compared with the corresponding values of the control group. Favorably, co-ingestion of CEE in line with OXP injection significantly ameliorated the OXP-induced deteriorations in the mentioned parameters.

Similarly, Table 2 shows a significant decrease in serum total protein and albumin levels was noticed post-OXP intoxication compared with the control group. Interestingly, administration of rats with CEE besides OXP injection markedly upregulated serum total proteins and albumin levels close to values of the normal group compared withOXP-intoxicated rats.

The obtained results of the OXP-intoxicated group showed a significant increase in total cholesterol, triglycerides, LDL-cholesterol level coupled with a significant decrease in HDL-cholesterol when compared with the control group. Interestingly, treatment of rats with OXP in line with CEE markedly ameliorated serum levels of cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol compared with OXP animals (Table 3).

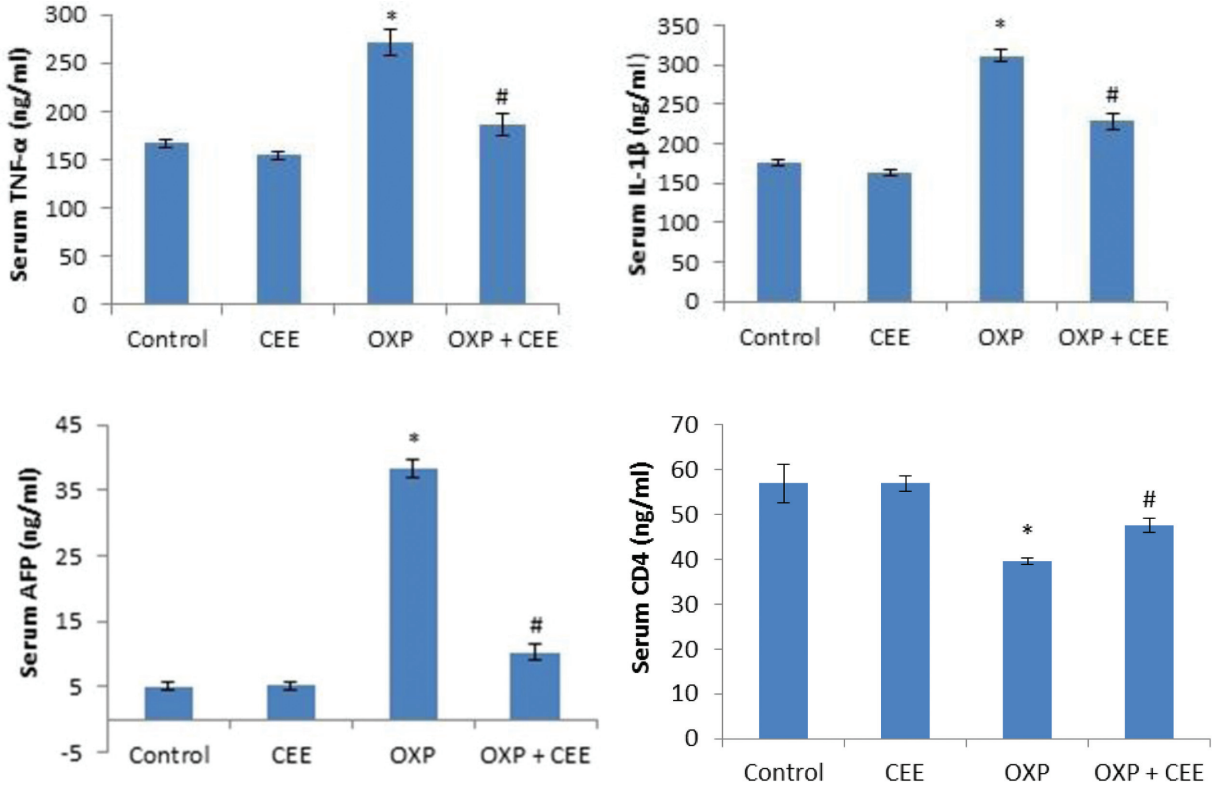
Table 3 shows that intoxication of rats with OXP led to a significant elevation in the levels of hepatic MDA and NO matched with a marked drop in GSH, SOD, and CAT values compared with the control group. Promisingly, treatment of animals with CEE besides OXP injection showed a significant decrease in hepatic MDA and NO levels coupled with a marked restoration in GSH, SOD, and CAT values compared with the OXP group (Table 4).

OXP-intoxicated treatment showed a significant increase in the percent of DNA fragmentation as compared with the control group, whereas treatment of OXP-intoxicated animals with CEE resulted in a significant improvement in DNA fragmentation percentage close to that of the control group (Fig. 5). Finally, Figs 6–11 describe and illustrate histopathological examinations of the liver sections of the study groups.

Discussion

OXP, like chemotherapeutic agents, has been used broadly in the treatment of various cancers and some

Figure 4



Serum TNF-α, IL-1β, CD4, and AFP levels of control, OXP-intoxicated and CEE-treated male albino rats. *Significantly different from the control group, while #significantly different from the OXP-intoxicated group ($P \leq 0.05$). CEE, costus ethanolic extract; AFP, alpha-fetoprotein; IL-1β,, Interleukin -1 Beta; OXP, Oxaliplatin; TNF-α, tumor necrosis factor-alpha.

Table 2 Markers of liver function of control, Oxaliplatin-intoxicated and costus ethanolic extract-treated male albino rats

	Control	CEE	OXP	OXP with CEE
ALAT (U/l)	38.3±2.4	35.1±3.9	131.1 ±8.1*	46.5±2.8#
ASAT (U/l)	35.4±2.9	32.8±6.2	117.9 ±7.7*	48.6±4.1#
GGT (U/l)	49.6±5.9	48.1±4.6	94±5.5*	76.8±6.3#
ALP (U/l)	178.8±9.4	170.3 ±10.3	256.6 ±21.5*	134±11.5#
Albumin (g/dl)	5.22±0.22	5.14 ±0.87	2.6±0.32*	4.9±0.17#
Total protein (g/dl)	9.4±0.72	9.47±.69	5.5±0.63*	8.35±0.74#

ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferases; CEE, costus ethanolic extract; OXP, Oxaliplatin. Data are presented as mean±SEM. Data were subjected to one-way analysis of variance followed by post hoc (Tukey) test at *P* value less than or equal to 0.05. *Significantly different from the control group, while #Significantly different from OXP.

Table 3 Serum cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol levels of control, Oxaliplatin-intoxicated and costus ethanolic extract-treated male albino rats

	Control	CEE	OXP	OXP with CEE
Cholesterol (mg/dl)	96.2 ±3.4	93.7 ±4.5	163.8 ±5.2*	118.8±6.1#
Triglycerides (mg/dl)	106.5 ±4.1	102.7 ±6.1	141.4 ±5.0*	114.1±4.8#
LDL-cholesterol (mg/dl)	37.0 ± 1.5	41.0 ±2.1	28.3±± 1.8*	38.7±0.7#
HDL-cholesterol (mg/dl)	74.0 ±4.6	73.7 ±4.5	163.7 ±6.0*	95.98±5.4#

CEE, costus ethanolic extract; OXP, Oxaliplatin. Data are presented as mean±SEM. Data were subjected to one-way analysis of variance followed by post hoc test (Tukey) test at *P* value less than or equal to 0.05. *Significantly different from the control group. #Significantly different from the OXP group.

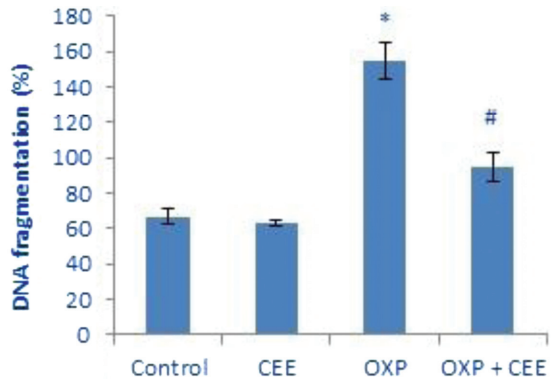
inflammatory diseases. One of the serious adverse effects of OXP is hepatotoxicity; approaches to reduce this complication are valuable in order to improve the quality of life of patients, and to ensure that treatment is more successful [24]. In the present study, OXP-induced hepatotoxicity is evident by the markedly increased activities of serum ALAT, ASAT, ALP, and GGT along with the declined albumin levels. These findings are in agreement with some recent studies that demonstrate increased liver marker enzymes in the serum of OXP-intoxicated rats [25,26].The elevated serum enzymes might be attributed to the increase of oxidative stress as a consequence of triggering ROS formation as a consequence to OXP. Moreover, it has been detected that OXP-induced toxicity is associated with an increase in lipid peroxidation, which is one of

Table 4 Hepatic values of malondialdehyde, nitric oxide, reduced glutathione, superoxide dismutase, and catalase of control, Oxaliplatin-intoxicated and costus ethanolic extract-treated male albino rats

	Control	CEE	OXP	OXP with CEE
MDA (μmol/g tissue)	270.2 ±9.5	292.9 ±10.3	544.6 ±21*	438.5±19#
NO (μmol/g tissue)	633±27	623±28	1577 ±54*	848.8±45#
GSH (nmol/g tissue)	625±29	661±34	325.6 ±21*	506±34#
SOD (U/g tissue)	149±12	152±14	53±4.4*	97±7.4#
CAT (U/g tissue)	17.3 ±0.9	18.9±1.1	8.1±0.5*	13.6±0.7#

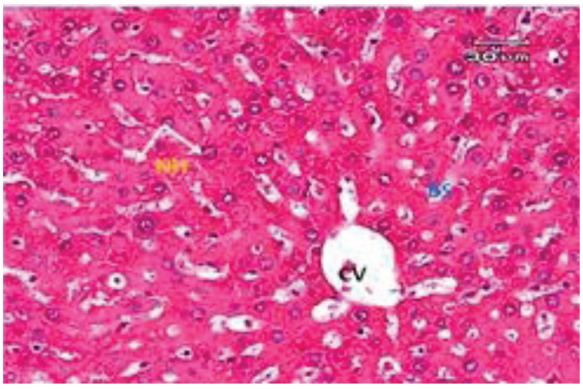
CAT, catalase; CEE, costus ethanolic extract; GSH, glutathione; MDA, malondialdehyde; NO, nitric oxide; OXP, Oxaliplatin; SOD, superoxide dismutase. Data are presented as mean±SEM. Data were subjected to one-way analysis of variance followed by post hoc (Tukey) test at *P* value less than or equal to 0.05. *Significantly different from the control group. #Significantly different from the OXP group.

Figure 5



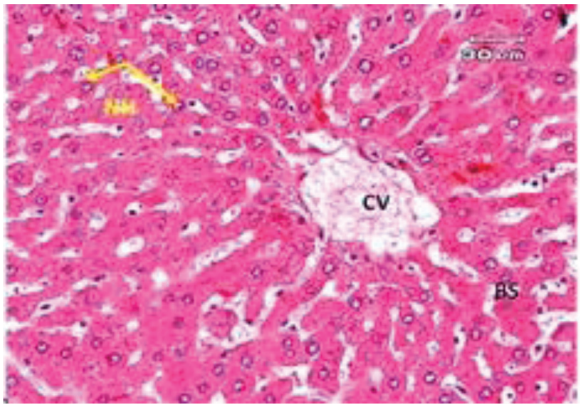
Percentage of hepatic DNA fragmentation of control, OXP-intoxicated and CEE-treated male albino rats. *Significantly different from the control group, while #significantly different from the OXP group. CEE, costus ethanolic extract; OXP, Oxaliplatin.

Figure 6



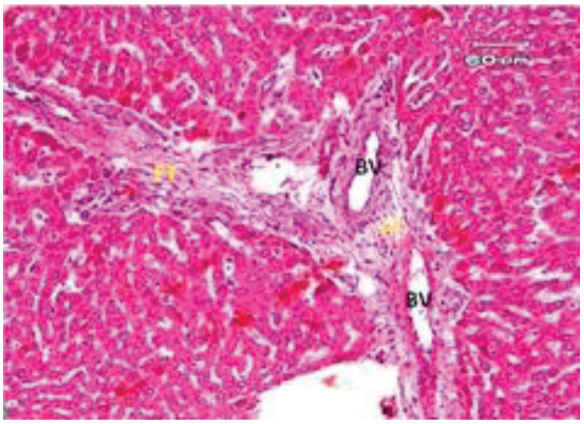
Photomicrograph of a liver section of a control rat showing the normal appearance of hepatocytes (NH); note the central vein (CV) and normal blood sinusoids (BS).

Figure 7



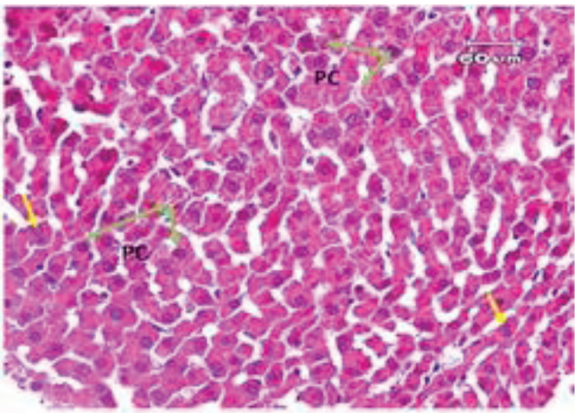
Photomicrograph of a liver section of a rat treated with CEE showing normal hepatic architecture (NH); note the central vein (CV) and blood sinusoids. CEE, costus ethanolic extract.

Figure 8



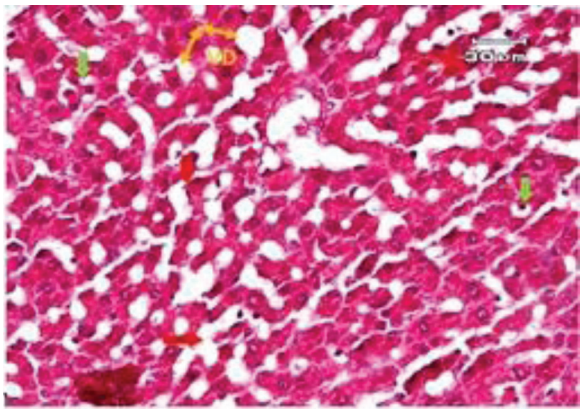
Photomicrograph of a liver section of Oxaliplatin-intoxicated rat showing the fibrous tissue (FT), fibrous bands formed of many fibroblasts, and collagen fibers; the bands run in septa between the hepatocyte lobules and around the blood vessels (BV).

Figure 9



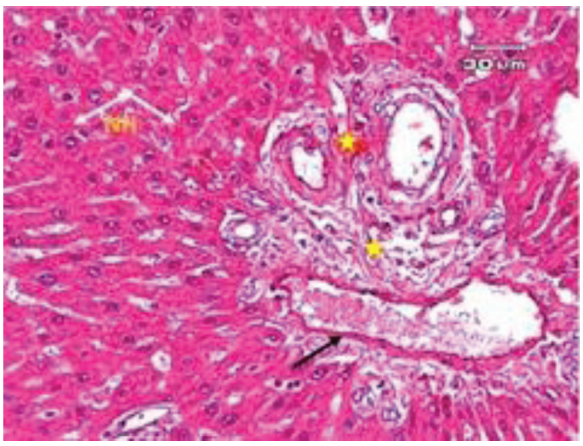
Photomicrograph of a liver section of Oxaliplatin-intoxicated rat (second filed) showing signs of degeneration in the form of pyknotic (P) nuclei (PC) and karyorrhexis (yellow arrow).

Figure 10



Photomicrograph of a liver section of Oxaliplatin-intoxicated rat (third filed) showing massive vacuolar (VD) and fatty changes (green arrow) and dilated blood sinusoids (red arrow).

Figure 11



Photomicrograph of a liver section of rats treated with Oxaliplatin combined with CEE showing the normal architecture of hepatocyte (NH), and thick fibrous band (star), formed of many fibrous bands and collagen around the blood vessel (arrow). CEE, costus ethanolic extract.

the most important destructive elements damaging cell membrane in many organs such as the liver and kidneys [24,27]; OXP-induced hepatic damage associated with progressive inflammation is referred to as chemotherapy-associated steatohepatitis [28].

In this study, CEE showed a hepatoprotective effect against OXP-induced liver damage as it succeeded to efficiently restore OXP-induced elevation of serum AST, ALT, GGT, and ALP activities. It was stated that natural antioxidants play a major role in reducing the oxidative stress through scavenging the excess free radicals [29], and CEE is one of the antioxidant-rich medicinal plants. Moreover, many authors have reported that the roots of this plant possess a cortisol-lowering effect [30]. Costunolide and dehydrocostuslactone, two natural sesquiterpene lactones, present in costus may play some pivotal roles through conjugation with mercapto (SH)-groups of target proteins to intervene in some key biological processes in cells [31] as they possess anti-inflammatory [32], anticancer [33], antiviral [34], antimicrobial [35], antifungal [36], antioxidant [37], antidiabetic [38], antiulcer [39], and hepatoprotective properties [30]. In this study, triglycerides, the main form in which fat is stored in the body, have been shown to be reduced by the CEE indicating CEE-protective effect against cardiovascular disease since this result goes in line with the observation of Duze *et al.* [40]. The increased serum HDL-cholesterol level observed in our study confirmed that effect as it is considered one of the strongest predictors of coronary heart disease (CHD) [41]. Although the mechanism of hypolipidemic effect of this extract is not yet known, it may however be attributed to its phytochemical constituents inherent that may have reduced blood lipids by competing with cholesterol biosynthesis in the liver and inhibiting the key enzyme hydroxyl-methyl-glutaryl coenzyme at the regulatory site.

The present study demonstrated that OXP-induced chronic oxidative stress in the hepatic tissues of intoxicated rats as confirmed by the significant increase of hepatic MDA and NO levels and reduction in the antioxidative battery (GSH, SOD, and CAT) can directly promote cell necrosis and activate the apoptotic pathway [42]. Excessive amounts of ROS may exert direct deleterious effects on cells through lipid peroxidation, protein degradation, and DNA damage [43], which evidenced herein by way of the elevated DNA damage percentage. Interestingly, CEE succeeded to protect against OXP as it markedly improved the radical scavenging activity, and hence inhibited oxidative stress progression. Restoration of GSH has a multifaceted role in antioxidant defense both as a direct scavenger of free radicals and as a co-substrate for peroxide detoxification by GSH peroxidases [44]. Also, SOD and CAT function in a sequential cascade manner in the antioxidant defense system. As an antioxidant enzyme, SOD catalyzes the removal of superoxide radicals generated from the oxidation of a singlet oxygen species. The end product of SOD action is hydrogen peroxide, which is an inhibitor of SOD if allowed to accumulate. Hydrogen peroxide is also a substrate for the production of hydroxyl radicals through the Fenton reaction cycle; hence this is the importance of CAT in the breakdown of hydrogen peroxide as it is formed of water and oxygen [45]. In this way, CEE causes activation of SOD and CAT function in protecting the cell from oxidative stress [46,47].

AFP gene is reactivated during hepatocarcinogenesis; cytoplasmic AFP enhances the proliferation of malignant hepatocytes, while extracellular AFP accelerates the growth of malignant hepatocytes through AFP receptors [48]. Besides hepatocytes, liver progenitor cells also develop AFP during their cellular differentiation [49]. Elevation of serum AFP is indicative of the proliferation of liver progenitor cells as a response to chronic liver injury [50]. Our study showed a significant elevation in serum AFP and CD4 levels after injection with OXP compared with normal control rats; this result agrees with previous studies [51,52]. This may be attributed to the activation of AFP gene and elevation in its serum level. Helper CD4+ T cells play a role in adaptive immunity by conditioning the environment and modulating the activity of other immune cells through cytokine production [53]. In the same way, the levels of the hepatic inflammatory cytokines, TNF-α and IL-1β, were increased markedly after OXP injection. Both inflammatory cytokines have been shown to cause hepatocyte injury through triggering a potent cytotoxic immune response and cell death [54]. TNF-α acts as a pivotal mediator in the progression of acute liver injury; consequently, its overproduction activates caspase-3, a member of the family of cysteine proteases, which, in turn, triggers hepatocellular necrosis and the apoptotic pathway [55]. Excessive ROS generation activates the JNK and caspase pathways, ultimately leading to TNF-α-induced cell death [56]. Oxidative stress also promotes the migration of inflammatory cells across the endothelial barrier, leading to tissue injury [57]. Therefore, it is reasonable to hypothesize that oxidative stress, which is exacerbated by OXP, may contribute to the rapid increase in the production of inflammatory cytokines in rats after OXP intoxication further aggravating liver injury, which mostly could be mechanized through the activation of AFP gene and helper CD4 T cells. In a promising manner, treatment of rats with CEE besides OXP potentially reduced OXP-induced inflammation, as it valuably decreased the level of serum TNF-α, IL-1β, CD4, and AFP. These were released from activated macrophages at the site of inflammation and influence hepatic metabolism by upregulating acute-phase protein gene expression [58]; this anti-inflammatory effect suggests that CEE may have genetic and immunomodulatory properties. Phytochemical analysis of the crude CEE showed that its main chemical constituents are phenolics and flavonoids, which have antioxidant effects, and these results are in agreement with previous reports [59]. The biochemical findings of our study are matched with the histopathological one, which proved that OXP causes liver damage as evidenced by the observation of necrotic hepatocytes with small degeneration in the form of pyknotic nuclei, and karyorrhexis, a portal space with severe inflammation, dilated blood sinusoids, and hepatocytes surrounded by lymphocytic infiltration; these architectural deteriorations might be caused by the membrane-damaging potential of OXP metabolites through oxidative stress mechanisms. These pathological changes directly correlated with the deteriorated biochemical and inflammatory markers, and were supported by a recent study [25]. Favorably, CEE administration effectively alleviated the OXP-induced hepatic histopathological changes and seemed to protect the liver tissue from OXP-induced acute oxidative stress possibly through antioxidant activities [60]. It has been established that ROS are involved in inflammation [32] and the protective action of CEE against OXP-resultant hepatic damage could involve anti-inflammatory, anti-dyslipidemia, and antioxidant mechanisms related to scavenging activity of ROS produced by OXP.

Conclusion

In conclusion, our results indicate that CEE was able to significantly ameliorate OXP-induced liver damage through anti-inflammatory, anti-dyslipidemia, and antioxidant mechanisms of its inherited constituents. CEE might be effective against different illnesses associated with the liver and can help in the management strategy of long-term use of OXP to help relieve patients of pain.

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Conflicts of interest

There are no conflicts of interest.

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