Egypt. Acad. J. Biolog. Sci., 11(2): 69- 85(2019)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg

Protective Effect of Costus (*Saussurea Costus*) Ethanolic Extract on Oxaloplatin®-Induced Histological Changes and Hemato-Cardiotoxicity in Adult Male Albino Rats

Mahmoud Ashry[1*]
1-Zoology Department, Faculty of Science, Al-Azhar University, Assuit, Egypt.
E.Mail mahmoud_ashry20@yahoo.com

ARTICLE INFO
Article History
Received:19/10/2019
Accepted:05/12/2019

***Keywords*:**
Cardiotoxicity, Histology, hematopoietic, Oxaloplatin®, costus, Rats

ABSTRACT

Due to the cytotoxic properties of Oxaloplatin®, including hemato-cardiotoxicity, this study aimed to investigate the efficiency of costus ethanolic extract to ameliorate cardio-hemato-toxicity accompanied Oxaloplatin® therapy in male rats. After acclimatization, adult male Wistar albino rats (160-200g) were randomly divided into four groups (10 animals each); the first group included normal rats and served as control, the second group included normal rats orally administered with 50 mg/kg of costus ethanolic extract (CEE) for 6 weeks, the third group included rats intraperitoneally intoxicated with of Oxaloplatin® (10 mg/kg/week) for a similar period, and the fourth group included rats intoxicated with Oxaloplatin® 6 weeks as the same time orally ingested with CEE six weeks. The obtained results revealed that CEE markedly restored the cardiological and hematological deteriorations induced by Oxaloplatin®; this was evidenced by the significant reduction in cardiac MDA and NO coupled with marked elevation of cardiac GSH, SOD and CAT. Also, serum CK, LDH, TNF-α, IL-1β, cholesterol, triglycerides and LDL were markedly lowered with elevated HDL and CD4. Cardiac DNA fragmentation percentage was declined significantly. Hematological indices were markedly restored. Moreover, the histopathological findings showed marked regeneration. In conclusion, CEE exhibited anti-cardio-hematotoxicities properties that may be mechanized through the radical scavenging and antioxidant characteristics of its active constituent's especially high phenolic content; reflecting a promising potency of CEE as a cardio-hemato protective supplement.

INTRODUCTION

Many anticancer drugs exhibit potentially life-threatening effects on the immune and hematopoietic systems (Ryu *et al.*, 2007). The treatment by means of chemotherapy is effective for treating many cancerous tumors although it exhibits adverse effects on normal cells. In particular, this treatment may destroy hematopoietic stem cells, thereby inducing severe side effects, such as anemia and leukopenia.

Application of anticancer drugs causes toxic impacts on healthy cells alongside on tumor cells due to its toxic metabolites.

Oxaliplatin® is a third-generation platinum-based anticancer agent. It is the first-line therapy used for the treatment of colorectal cancer (Arnold *et al.,* 2017). The most common side effect of Oxaliplatin® is peripheral neuropathy, Nephrotoxicity, ototoxicity and cardiotoxicity are the known side effects of the drug which occurs in both acute and chronic forms (Argyriou, 2015). The most significant factors limiting its utilization are nephrotoxicity and cardiotoxicity that can occur acutely or cumulatively and by similar mechanisms (Varga *et al.,* 2015).

Cardiac injury has been evaluated by oxidative damage on Oxaliplatin®-induced heart tissue. While CK-MB is a well-known marker of cardiac injury, originates in cardiomyocytes (Donato 1999) and it is not clear whether its expression correlates with CKM-MB or can be useful as a new marker of cardio-oncology. Several studies have been performed about the cardioprotective effects of natural products and found as promising during cancer chemotherapy (Mojzisova *et al.,* 2006 & Vincent *et al.,* 2013).

Natural products are known to possess a wide range of biological activities. Flavonoids and polyphenolic compounds are the active antioxidant principles found in a large number of natural products (Enseleit *et al.,* 2013).

Saussurea costus, synonymous with Saussurea lappa Clarke (Parmar *et al.,* 2012) belong to the genus Asteraceae (Singh *et al.,* 2017) and is commonly known as Indian costus (Gwari *et al.,* 2013) or Kuth root (Amara *et al.,* 2017). It was used as a medicinal plant for the treatment of various ailments as asthma, inflammatory diseases, ulcer and stomach problems (Pandey *et al.,* 2007). Moreover, costus has been mentioned in Prophet's medicine for the treatment of many diseases (AL-Kattan, 2013) and was used in modern medicine (Amara *et al.,* 2017).

The Saussurea (costus) is one of the antioxidant-rich medicinal plants (Saleem *et al.,* 2013). The major components are sesquiterpene lactones such as costunolide and dehydrocostus lactone. It has various biological activities such as anti-inflammatory, immune-modulator, hypo-glycemic, anti-hepatotoxic, hypolipidemic, antiparasitic, antiviral and anticancer activities (Zahara *et al.,* 2014; Amara *et al.,* 2017). This study aimed to explore the ameliorative effects of costus ethanolic extract (CEE) against cardiotoxicity and hematopoietic toxicities complicated by Oxaliplatin ® therapy.

MATERIALS AND METHODS

Plant Materials 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 (TSN) TSN 780691. The ethanolic extract of dry powdered roots was carried out according to the modified method of Filipiak-Szok *et al.* (2017); in brief, 2 g of powder were soaked in 20 ml absolute ethanol at room temperature for 24 h 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. Extractions were performed in triplicate.

Determination of Total Extract Yield:

The combined extracts were transferred to a quick fit round bottom flask with known weight (W1), freeze-dried and weighed again (W2) and finally, the yield was calculated from the following formula:

$$\text{Extract yield (g/ g crude herb)} = (W2 - W1)/W3$$

Where,
W1 is the weight of clear and dry quick fit flask in grams,
W2 is the weight of the flask after lyophilization in grams
W3 is the weight of the crude powdered herb in grams

Determination of Total Phenolic Content:

Phenolic content of the CEE was performed by dissolving five 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. Estimation of phenolic compounds as catechin equivalents was carried out using a standard curve (Jayaprakasha and Jaganmohan, 2000).

DPPH Radical Scavenging Activity:

The capacity of antioxidants of CEE to quench DPPH radicals was determined as previously described (Nogala-Kalucka et al., 2005). 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 x 10^{-5}mol/L), 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 following equation:

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

Estimation of Reducing Power:

The reducing power of the used extract was determined according to the method described by Sethiya et al. (2014) with some modifications. From both extract and ascorbic acid, 0.5 ml of different concentrations (50, 100, 200, 400 and 800µg /ml) 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 50ºC in a water bath for 20 minutes; 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.

HPLC Analysis of Phenolic Constituents*:*

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil C18 column (4.6 mm x 250 mm i.d., 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 multi-wavelength 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*:*

Male albino rats (160-200g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt; the animals were kept in suitable plastic cages and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental animals according to

the ethical committee of National Research Centre (FWA 00014747); however, this study was approved by the same ethical committee. After the animals were acclimatized with experimental room conditions, they were divided randomly into four groups (10 animals each). The first group included healthy animals that were fed a standard diet and intraperitoneally injected with 1 ml isotonic saline without any treatments and acting as normal control, the second group included healthy animals that were orally administered with 50 mg/kg/day of costus ethanolic extract (CEE) consecutively for six weeks, the third group included animals that intraperitoneally injected with 10 mg/kg/week of Oxaloplatin® for six weeks and acting as positive control; and fourth group include animals that intoxicated six weeks with Oxaloplatin® companied with orally administrated daily with CEE for six weeks.

Blood and Tissue Sampling*:*

At the end treatment period, rats fasted overnight, and following anesthesia with diethyl ether, 3-5 ml blood samples were withdrawn from the retro-orbital plexus using heparinized-sterile glass capillaries. Each blood sample was divided into 2 portions: the smaller portion was taken on heparin for the determination of hematological parameters, while the other portion was coolly centrifuged at 3000 rpm for 15 minutes and the sera were separated and stored at -80°C until biochemical determinations as soon as possible. After blood collection, the animals were sacrificed soon, and then the heart of each animal was dissected out, washed in saline, dried, rolled in a piece of aluminum foil and stored at -80 °C for homogenization and biochemical determinations and cardiac DNA fragmentation percentage. The heart organ was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); the homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions; the supernatant was divided into aliquots and stored at -80°C till the determination of the oxidative stress markers in the cardiac tissue.

Assessment of Complete Blood Count:

Cell blood counter (full automatic –Model PCE – 210 N, Japan) was used for measuring of red blood corpuscles (RBCs) count ($10^6/cm^3$), Hemoglobin (Hb) content (g/dl), hematocrit (Hct) percentage, mean corpuscular volume (MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl), platelets (PLT) count ($10^3/cm^3$), and total leucocytes count (TLC) count ($10^3/cm^3$).

Biochemical Determinations*:*

All the biochemical measurements were carried out using Schimadzu spectrophotometer (UV–vis 1201, Japan). Serum ALAT and ASAT activities were determined using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostica mbH, Germany. Lipid profile was estimated using kits purchased from DiaSys Diagnostic systems GmbH Germany. LDH and CK-MB activity was assayed colorimetrically using reagent kits purchased from BioVision, South Milpitas, California, USA.

Oxidative Stress Markers of Heart Tissue*:*

Cardiac GSH and NO levels as well as SOD and CAT activities were estimated using kits obtained from Biodiagnostic, Dokki, Giza, Egypt.

Determination of TNF-A, IL 1β and CD4*:*

Using ELISA (Dynatech Microplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada), serum TNF-α, IL 1β and CD4 concentrations were measured using rat elisa kits (SG-10057, SG-10179 and SG-10127, respectively) purchased from SinoGeneClon Biotech

Co., Ltd, No.9 BoYuan Road, YuHang District 311112, Hang Zhou, China.

Determination of Cardiac MDA*:*

Cardiac MDA (indirect index for lipid peroxidation) level was determined chemically according to the method described by Ruiz-Larnea *et al.* (1994). In this method, 0.5 ml heart homogenates' supernatant was added to 4.5 ml working reagent [0.8 g thiobarbeturic acid dissolved in 100 ml perchloric acid 10%, and mixed with TCA (20%) in a volume ratio 1: 3, respectively]; then in boiling and shaking water bath, the sample-reagent mixture was placed for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm. Finally, the absorbance of the clear pink supernatant was measured at 535 nm against the reagent blank [0.5 ml distilled water + 4.5 ml working reagent]. Cardiac MDA level (nmol/g tissue) was calculated according to the formula below.

MDA (nmol/g tissue) = [{$A_{535}\times 10^9$ / $(1.56 \times 10^5) \times 10^3$} x AD] × 10.

Where 1.56x105 $M^{-1}L^{-1}cm^{-1}$ is MDA extinction coefficient and AD is assay dilution (10).

Cardiac DNA Fragmentation Percentage:

The degree 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 (Perandones *et al.*, 1993). The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet. The cardiac tissues were lysed in 0.5 ml of hypotonic lysis buffer containing 10 mM Tris–HCl (pH 8), 1 mM EDTA and 0.2% Triton X-100, and centrifuged at 14,000 ×g for 20min at 4 °C. The pellets were resuspended in a hypotonic lysis buffer. To the resuspended pellets and the supernatants, 0.5 ml of 10% trichloroacetic acid was added. The samples were centrifuged for 20 min at 10, 000 ×g at 4 °C, and the pellets were suspended in 500 µl of 5% TCA. Subsequently, each sample was treated with a double volume of diphenylamine (DPA) solution [200mg DPA in 10 ml glacial acetic acid, 150µl of sulfuric acid and 60µl acetaldehyde] and incubated at 4°C for 48h. The proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the following equation:

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

Histopathology:

Paraffin sections of 5µm thick were stained with haematoxlin and eosin (Drury and Wallington, 1980) and investigated by a light microscope.

Statistical Analysis:

Comparisons between means were carried out using one-way analysis of variance (ANOVA) followed by a post hock (Tukey) multiple comparisons test at $p \leq 0.05$ according to Steel and Torrie (1960). This was carried out using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

RESULTS

The yield, total phenolic content (TPC) and radical scavenging activity (RSA) and reducing the power of the *Costus* (*Saussurea costus*) ethanolic extract (CEE) are shown in figures (1 and 2). As shown in figure (3) 16 phenolic compounds were identified in CEE using HPLC analysis. The compounds identified were found to include high contents of naringenin, chlorogenic cid, ferulic acid, taxifolin, gallic acid, coffeic acid (Table 1).

A significant drop was noticed in Hb, HCT levels; RBC, WBCs, and platelets count; and blood indices (MCV, MCH, MCHC) as a consequence of OXP intoxication in comparison to the healthy group. Favorably, administration of OXP-pretreated rats with CEE resulted in a

marked improvement in the mentioned hematological parameters close to the healthy values (Table 2).

Table (3) indicated that OXP intoxication induced significant elevations in serum ASAT and ALAT activities in comparison to the control group. Meanwhile, treatment of OXP-intoxicated animals with CEE significantly restored the mentioned liver enzymes

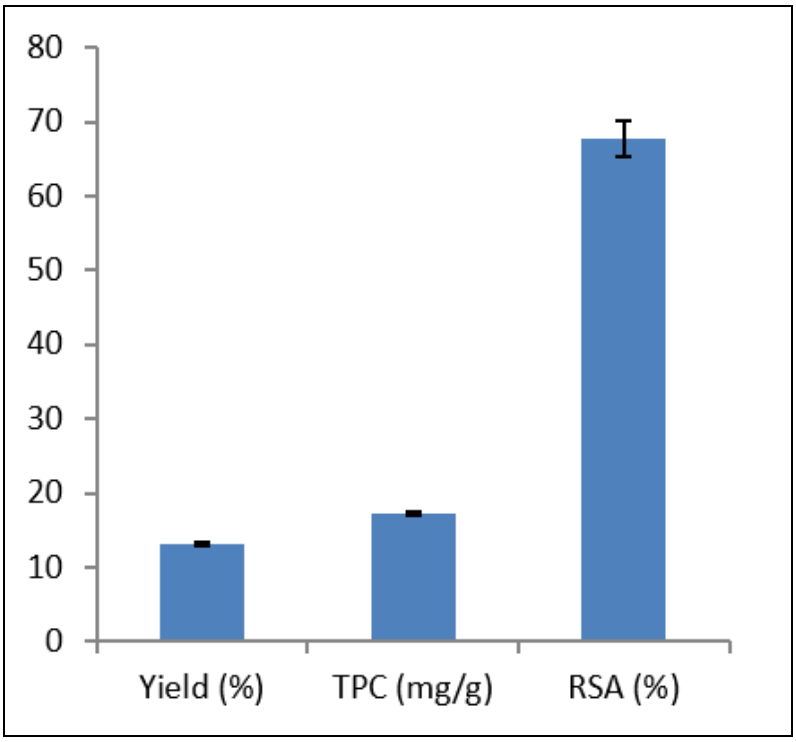

Fig. 1. Yield (%), total phenolic content (mg /g) and radical scavenging activity (%) of dry powdered roots of *Costus* (*Saussurea costus*) ethanolic extract.

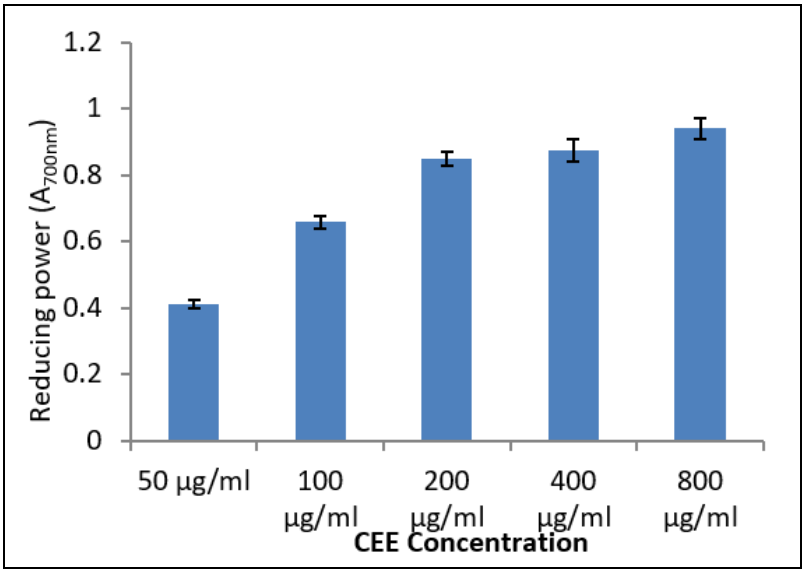

Fig.2. Reducing power of *Costus* (*Saussurea costus*) ethanolic extract.

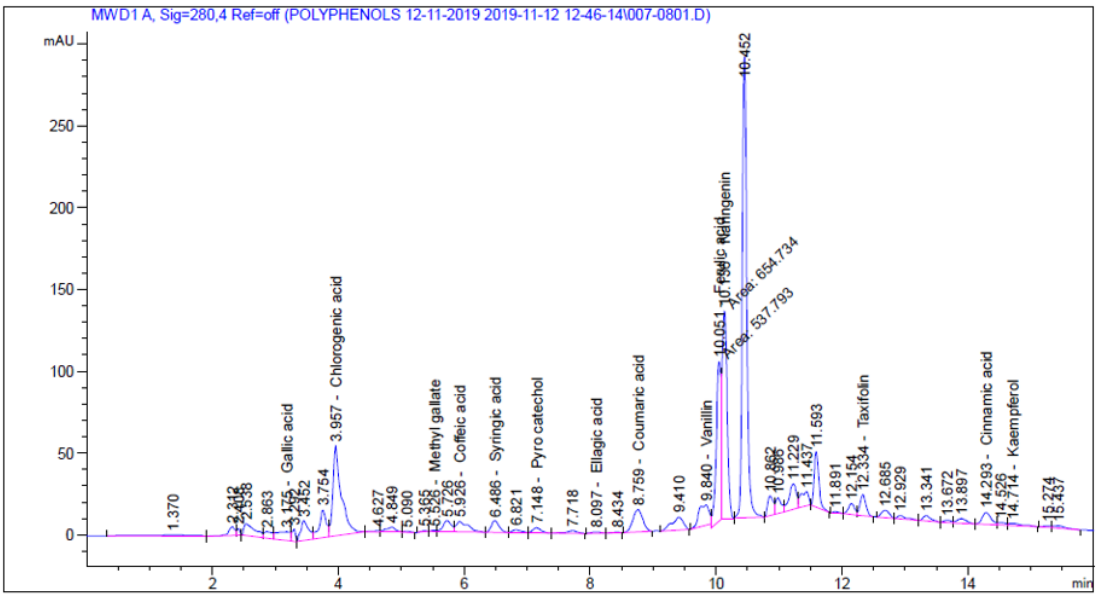

Fig. 3. HPLC analysis of phenolic constituents *Costus* (*Saussurea costus*) ethanolic extract.

Table 1: Phenolic constituents of the ethanolic extract of *Saussurea costus* using HPLC analysis.

	Area	Conc. (µg/ml = µg/ 6.8mg)	Conc. (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
Coffeic 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

Table 2. Effect of Oxaloplatin® and CEE on the level of whole blood indices.

	Control	CEE	OXP	OXP with CEE
Hb (g/dL)	15.1±0.55	15.4±0.45	9.5±0.32*	14.55±0.2#
HCT (%)	58.5±2.95	56.5±2.33	32.7±0.88*	54.05±0.37#
RBC (10^6/uL)	7.82±0.33	7.73±0.19	4.66±0.06*	6.65±0.14#
MCV (fl)	74.9±1.59	80.167±1.1	64.05±1.53*	73.45±0.02#
MCH (pg)	19.36±0.09	20.9±0.62	13.96±0.61*	20.7±0.4#
MCHC (g/dL)	25.93±0.55	26.06±0.41	21.06±0.62*	26.8±0.23#
WBC (10^3/ uL)	8.1±0.49	510±83.08	2.33±0.44*	6.25±0.31#
PLT (10^3/uL)	623±64.42	8.2±0.38	140±69.87*	235.28±133.2#1

Data are presented as mean ±standard error and subjected to one-way ANOVA followed by a post hoc test (Duncan) at $p \leq 0.05$. OXP is Oxaloplatin®; CEE is costus ethanolic extract; (*) is significantly different from the control group; (#**)** is significantly different from OXP group.

Table 3. Effect of Oxaloplatin®and CEE on serum ALAT and ASAT activity.

	Control	CEE	OXP	OXP with CEE
ALAT (U/L)	45.2±4.4	44.1±5.1	146.1±12.1*	84.5±9.8#
ASAT (U/L)	51.4±5.9	52.8±7.2	175.9±13.7*	92.6±7.1#

Data are presented as mean ±standard error and subjected to one-way ANOVA followed by a post hoc test (Duncan) at $p \leq 0.05$. OXP is Oxaloplatin®; CEE is costus ethanolic extract; (*) is significantly different from the control group; (#**)** is significantly different from OXP group.

Unlikely, OXP-intoxication led to a significant increase in serum cholesterol, triglycerides and LDL level coupled with a significant decrease in HDL when compared with the control group. Interestingly, administration of OXP-intoxicated rats with CEE resulted in a marked improvement in lipid profile parameters monitored from the significant decrease in cholesterol, triglycerides and LDL, and the marked increase in the HDL level compared to OXP-treated animals (Table 4).

Table 4. Effect of Oxaloplatin®and CEE on serum cholesterol, triglycerides, LDL and HDL levels

	Control	CEE	OXP	OXP with CEE
CHO (mg/dl)	125±5.4	122±7.5	245.8±5.2*	165.8±9.1#
TRG (mg/dl)	132.5±8.1	141.7±7.1	276.4±9.0*	192.1±8.8#
LDL-C (mg/dl)	40.0± 1.5	41.0±3.1	27.3± 1.8*	35.7±1.5#
HDL-C (mg/dl)	81.2±4.6	83.7±4.5	188.7±6.0*	106.98±5.4#

Data are presented as mean ±standard error and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. OXP is Oxaloplatin®; CEE is costus ethanolic extract; (*) is significantly different from the control group; (#**)** is significantly different from OXP group.

In respect to Table (5), OXP-intoxication induced a marked deterioration in the cardiac oxidative stress status; evidenced by the significant increase in cardiac MDA and NO values coupled with a significant reduction in the activity of SOD and CAT as well as GSH level. In a favorable manner, CEE-treatment of animals intoxicated with OXP led to a significant drop in cardiac MDA and NO levels combined with a marked rise in GSH level, and SOD and CAT activities in comparison to the OXP-intoxicated group.

Table 5. Effect of Oxaloplatin® and CEE on cardiac MDA, NO, GSH, SOD and CAT.

Oxidative stress	Control	CEE	OXP	OXP with CEE
MDA (µmol/g tissue)	125±3.5	122.4±4.2	352.1±11.4*	242.1±14.5#
NO (µmol/g tissue)	265.4±12.5	249.5±15.1	752.4±24*	424.8±21.1#
GSH (nmol/g tissue)	445.2±19	452±24	215.4± 16*	351±26#
SOD (U/g tissue)	92.4±9.4	102±12	46.2±4.4*	70.5±4.4#
CAT (U/g tissue)	10.3±0.9	9.9±1.1	3.55±1.5*	6.6±1.22#

Data are presented as mean ±standard error and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. OXP is Oxaloplatin®; CEE is costus ethanolic extract; (*) is significantly different from the control group; (#**)** is significantly different from OXP group.

The obtained data showed that a significant increase in serum CK and LDH activity in OXP group when compared with the control group. Interestingly, administration of OXP rats with CEE improved serum CK and LDH activity within normal values as it significantly decreased CK and LDH activity compared to OXP animals, data in figure (4).

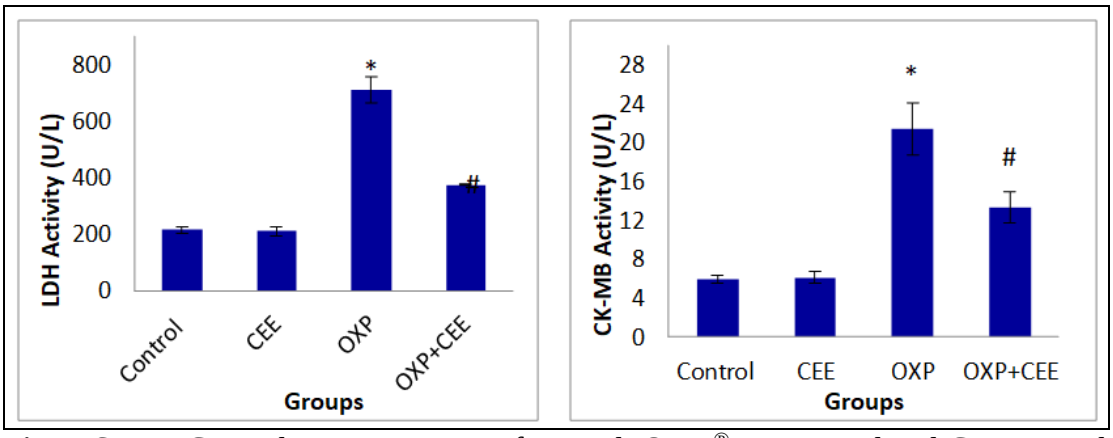

Fig 4: Serum CK and LDH activity of control, OXP ®-intoxicated and CEE-treated male albino rats. * is significantly different from the control group, while # is significantly different from OXP group ($p \leq 0.05$).

The obtained results showed significant increases in TNF-α, IL1β and cardiac DNA fragmentation level coupled with significant decreases in CD4 in OXP group when compared with the control group. Interestingly, administration of OXP rats with CEE improved all inflammatory cytokine and cardiac DNA fragmentation within normal values as it significantly decreased TNF-α, IL1β and DNA fragmentation while significantly increasing the CD4 level compared to OXP animals (Fig. 5).

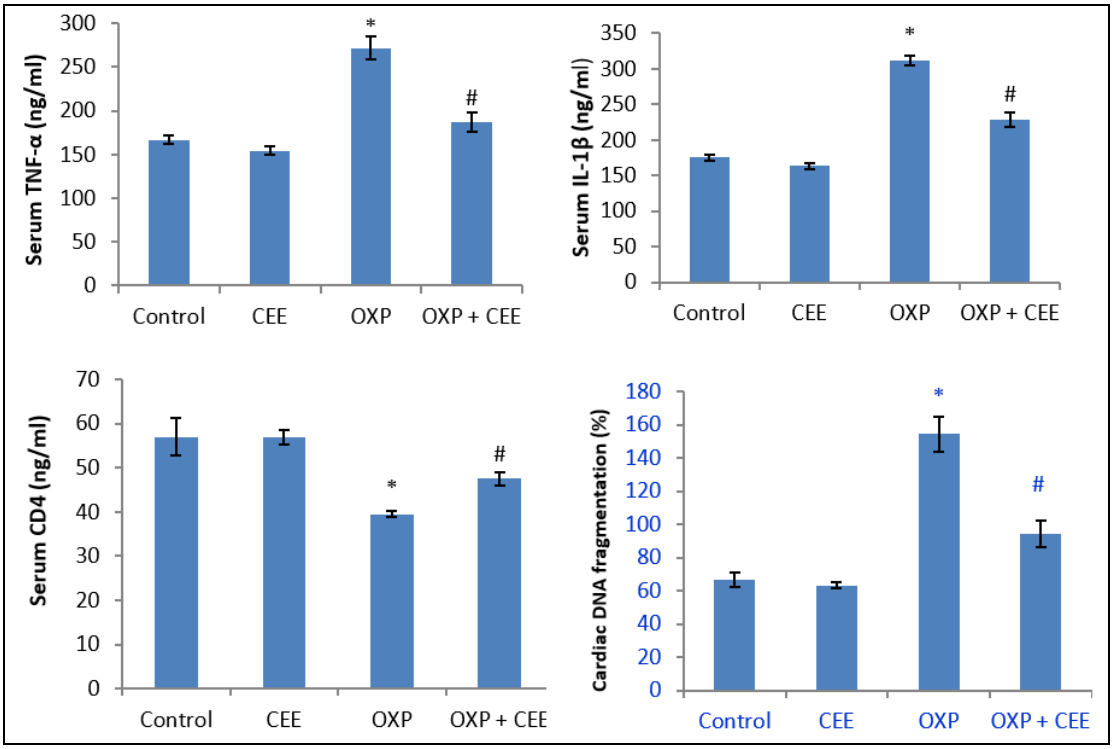

Fig 5. Serum TNF-α, IL1β, CD4 and cardiac DNA fragmentation percentage of control, OXP ®-intoxicated and CEE -treated male albino rats. * is significantly different from the control group, while # is significantly different from OXP group ($p \leq 0.05$).

Histopathological Examination:

The microscopic examinations of the cardiac sections of the normal, Oxaloplatin®-intoxicated and CEE-treated male rats are illustrated in the figures (6-9).

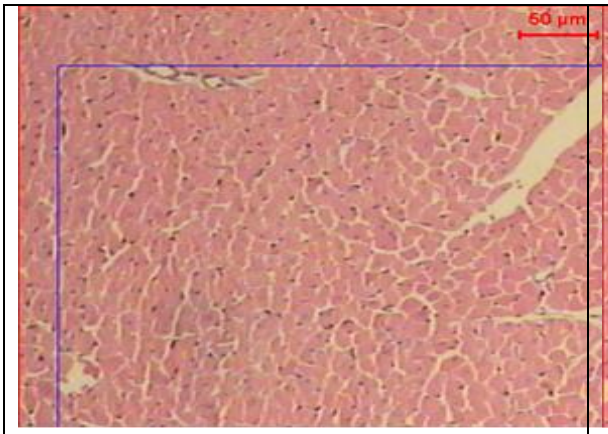

Fig. 6: A photomicrograph of cardiac myocytes, control group myocytes show normal morphology (H&E, X100).

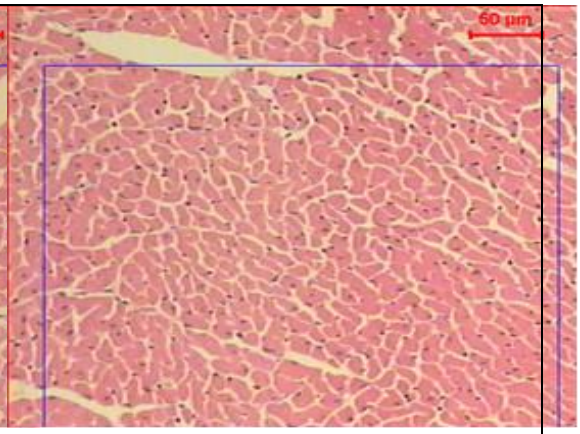

Fig. 7: A photomicrograph of cardiac myocytes for CEE group showing normal morphology (H&E, X100).

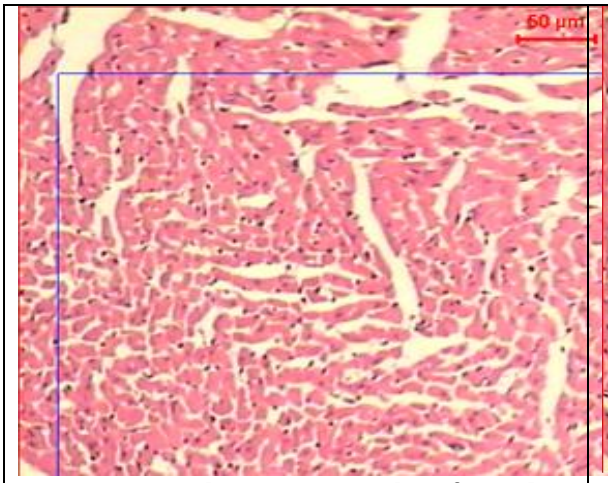

Fig. 8: A photomicrograph of cardiac myocytes for Oxaloplatin® group showed focal rarefactions (H&E, X100).

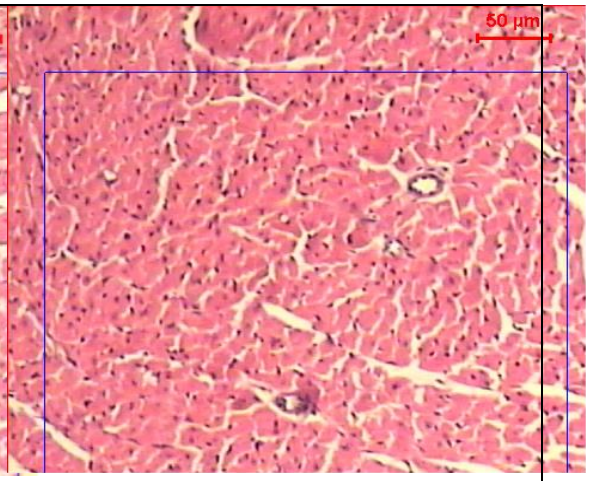

Fig. 9: A photomicrograph of cardiac myocytes for Oxaloplatin® companied with CEE group myocytes showed normal morphology (H&E, X100).

DISCUSSION

Oxaloplatin® (OXP) is a cytotoxic drug that is highly effective in the treatment of various human cancers particularly colorectal cancer and some types of leukemia and autoimmune diseases. The clinical utility of the platinum agent has been hampered by dose-limiting toxicities; one of the most frequent complications is myocarditis (Varga *et al.,* 2015). This study aimed to investigate the ameliorating potential of CEE on hemato-cardiotoxicities accumulated as a consequence of Oxaloplatin®-therapy in rats.

In the present study, Oxaloplatin® administration significantly increased the activities of serum LDH, CK-MB, ASAT and ALAT; this result is in accordance with the studies of Eryılmaz *et al.* (2017). It was stated that Oxaloplatin®is a cardiotoxic agent inducing a direct myocardial endothelial damage and destruction of myocardial cells; thus LDH, CK-MB and ASAT were raised. Similarly, OXP-induced hepatotoxicity is evident by the markedly increased activities of serum ALAT and ASAT 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 the most important destructive elements damaging cell membrane in many organs such as the liver, kidney and heart (Bano and Najam, 2016 & Demirci *et al.,* 2019); OXP-induced hepatic damage associated with progressive inflammation is referred to as chemotherapy-associated steatohepatitis (Schwingel *et al.,* 2014).

In the present study, costus ethanolic extract (CEE) showed a cardio-protective effect against OXP-induced cardiac damage as it succeeded in efficiently restoring the OXP-induced elevation of serum LDH, CK-MB, AST and ALT, activities. It was stated that natural antioxidants play a major role in reducing oxidative stress via scavenging the excess free radicals (Ahmad *et al.,* 2012), 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 (Ambavade *et al.,* 2009). 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 (Jeong

et al., 2007) as they possess anti-inflammatory (Butturini *et al.,* 2014), anticancer (Kuo *et al.,* 2009), antiviral (Chen *et al.,* 1995), antimicrobial (Lee *et al.,* 2014), antifungal (Barrero et al., 2000), antioxidant (Seo et al., 2012), antidiabetic (Upadhyay *et al.,* 1996), antiulcer (Sutar *et al.,* 2011), and cardio-protective effects (Ambavade *et al.,* 2009). In the present study, triglycerides, the main form of which fat is stored in the body, has been shown to be reduced by the CEE indicating CEE- protective effect against the cardiovascular disease since; this result goes in line with the observation of Duze *et al.* (2012); the increased serum HDL-cholesterol level observed in our study confirmed that effect, since it is considered one of the strongest predictors of CHD (Maria & Roland, 2006). In spite 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 co-enzyme at the regulatory site.

Intoxication with Oxaloplatin® led to significant deteriorations in all hematological measurements monitored from the reduced RBCs count, hematocrit percentage, MCV, MCH and MCHC leading to a condition of hypochromicmicrocytic anemia that resulted in a dependent degree of tissue hypoxia, especially heart and brain tissues. This was in accordance with Ito *et al.* (2018) who reported that Oxaloplatin® exhibiting adverse effects on blood and bone marrow due to the interaction of its active metabolites with the hematopoietic tissues which subsequently cause depression of the hematopoietic activities. Furthermore, Oxaloplatin®caused decrease in these measurements due to immunosuppressive effect of Oxaloplatin®and retarded haematopoeisis and /or interaction of Oxaloplatin® active metabolic with proteins of the erythrocytes membranes leading to elevation of RBCs destruction rate and decrease in Hb synthesis; hence decline in MCV (microcytosis), MCH and MCHC (hypochromasia) (Ito *et al.,* 2018) resulting finally in a case of hypochromic-microcytic anemia which mainly a manifestation of iron-deficiency. It could be seated that Oxaloplatin® may inhibit either absorption or assimilation of iron.

The present study demonstrated that Oxaloplatin® induced chronic oxidative stress in the cardiac tissues of intoxicated rats as confirmed by the significant increase of cardiac MDA and NO levels and reduction in the anti-oxidative battery (GSH, SOD and CAT) those can directly promote cell necrosis and activate the apoptotic pathway (Koek *et al.,* 2011). Excessive amounts of ROS may exert direct deleterious effects on cells through lipid peroxidation, protein degradation and DNA damage (Chen *et al.,* 2013) which is evidenced herein via the elevated DNA damage percentage. Interestingly, CEE succeeded to protect against Oxaloplatin® as it markedly improved the radical scavenging activity, and hence inhibited the 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 glutathione peroxidases (Ewis and Abdel-Rahman, 1995). 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 radical through the Fenton reaction cycle; hence this is the importance of CAT in the breakdown of

hydrogen peroxide as it is formed to water and oxygen (Tao *et al.,* 2009). In this way, CEE activation of SOD and CAT function in protecting the cell from oxidative stress (Ha and Lee, 2005; Tchamgoue *et al.,* 2015).

In the same consequence, the levels of the serum inflammatory cytokines, TNF-α and IL-1β, were increased markedly after Oxaloplatin®injection. Both inflammatory cytokines have been shown to cause hepatocyte injury by triggering a potent cytotoxic immune response and cell death (Del campo *et al.,* 2018). As 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 (Gao, 2013). Excessive ROS generation activates the JNK and caspase pathways, ultimately leading to TNF-α-induced cell death (Deng *et al.,* 2003). Oxidative stress also promotes the migration of inflammatory cells across the endothelial barrier, leading to tissue injury (Van Wetering *et al.,* 2002). Therefore, it is reasonable to hypothesize that oxidative stress, which is exacerbated by Oxaloplatin®, may contribute to the rapid increase in the production of inflammatory cytokines in rats after OXP intoxication further aggravating the liver injury. By a promising manner, treatment of rats with CEE besides Oxaloplatin® potentially reduced OXP-induced inflammation, as it valuably decreased the level of serum TNF-α and IL-1β those were released from activated macrophages at the site of inflammation and influence hepatic metabolism by upregulating acute-phase protein gene expression (Maddux *et al.,* 2001); this anti-inflammatory effect suggest that the CEE may have 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 (Tag *et al.,* 2017). Histopathological investigations are in good agreement with biochemical changes; findings of the present study indicated that traditional plants extract showed a significant cardioprotective and curative activity against Oxaloplatin® induced cardiac injury. The chemical constituents of *Costus (Saussurea costus)* are responsible for their anti-inflammatory and cardioprotective activities; this could be performed through the antioxidant potentials of these constituents.

Conclusion

Costus (*Saussurea costus*) ethanolic extract may be as promising as hemato-cardio protection against OXP hemato-cardio toxicity through their antioxidant activity and radical scavenging activities.

REFERENCES

Ahmad, S., Khan, M.B., Hoda, M.N., Bhatia, K., Haque, R. and Fazili, I.S. (2012): Neuroprotective effect of sesame seed oil in 6-hydroxydopamine induced neurotoxicity in mice model: Cellular, biochemical and neurochemical evidence. *Neurochemical Research,* 37: 516-26.

AL-Kattan, M.O. (2013): Anti-bacterial effect of Indian costus and sea-qust and their water extracts on some pathogenic bacteria of the human respiratory system. *Journal of Medicinal Plants Research,* 7 (20):1418-1423

Amara, U., Mashwani, Z.R., Khan, A., Laraib, S., Wali, R. and Sarwar U. (2017): Conservation Status and Therapeutic Potential of Saussurea lappa: An Overview. *American Journal of Plant Sciences,* 8: 602-614

Ambavade, S.D., Mhetre, N.A., Tate, V.D. and Bodhankar, S.L.

(2009): Pharmacological evaluation of anxiolytic effect of aqueous extracts of Saussurea lappa roots in mice. *European Journal* of Integrative *Medicine*; 1: 131-137.

Argyriou, A.A. (2015): Updates on Oxaliplatin Induced Peripheral Neurotoxicity (OXAIPN).*Toxics;* 3: 187-197.

Arnold, M.; Sierra, M.S.; Laversanne, M. et al. (2017): Global patterns and trends in colorectal cancer incidence and mortality. *Gut;* 66(4): 683-691.

Bano, N. and Najam, R. **(**2019**):** Histopathological and biochemical assessment of liver damage in albino Wistar rats treated with cytotoxic platinum compounds in combination with 5-fluorouracil. *Archives of Medical Science; 15* (4): 1092–1103.

Barrero, A.F., Oltra, J.E., Alvarez, M., Raslan, D.S., Saude, D.A. and Akssira, M. (2000): New sources and antifungal activity of sesquiterpene lactones. *Fitoterapia,* 71: 60-64.

Butturini, E., Paola, R., Suzuki, H., Paterniti, I., Ahmad, A., Mariotto, S. and Cuzzocrea, S. (2014): Costunolide and dehydrocostuslactone, two natural sesquiterpene lactones, ameliorate the inflammatory process associated to experimental pleurisyinmice. *European Journal* Pharmacology, 730: 107-115.

Chen, H.C., Chou, C.K., Lee, S.D., Wang, J.C. and Yeh, S.F. (1995): Active compounds from *Saussurea lappa* Clarks that suppress hepatitis B virus surface antigen gene expression in human hepatoma cells. *Antiviral Research,* 27: 99-109.

Chen, Y., dong, H., Thompson, D.C., Shertzer, H.G., Nebert, D.W. and Vasiliou, V. (2013): Glutathione defense mechanism in liver injury: Insights from animal models. *Food and Chemical Toxicology,* 60: 38-44.

Del, C., José, A., Gallego, P. and Lourdes, G. (2018): Role of inflammatory response in liver diseases: Therapeutic strategies. *World journal of hepatology,* 10(1): 1.

Demirci, T., Gedikli, S., Ozturk, N. and Aydemir C.N. (2019): The Protective Effect of N-acetylcysteine Against Methotrexate-Induced Hepatotoxicity in Rat. *Eurasian Journal of Medical Investigation*, 3(3):219–226.

Deng, Y., Ren, X., Yang, L., Lin, Y. and Wu, X. (2003): A JNK-dependent pathway is required for TNFalpha-induced apoptosis. *Cell,* 115: 61-70.

Donato, R. (1999): Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. *Biochim Biophys Acta.* 1450: 191-231.

Drury, R.A.B. and Wallington, E.A. (1980): Preparation and fixation of tissues. In: Drury RAB, Wallington EA, editors. Carleton's Histological Technique. 5. Oxford: Oxford University Press; pp. 41–54.

Duze, B.N., Sewani-Rusike, C.R. and Nkeh-Chungag, B.N. (2012): Effects of an ethanolic extract of Garcinia kola on glucose and lipid levels in streptozotocin induced diabetic rats. *African Journal of Biotechnology,* 11 (33): 8309-8315.

Enseleit, F., Sudano, I., Periat, D., Winnik, S., Wolfrum, M. and Flammer, A.J. (2012): Effects of Pycnogenol on endothelial function in patients with stable

coronary artery disease: a double-blind, randomized, placebo-controlled, cross-over study. *European Heart Journal;* 33: 1589- 97.

Ewis, S.A. and Mohamed S.A. (1995): Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. *Journal of applied toxicology,* 15(5): 387-390.

Filipiak-Szok, A., Kurzawa, M., Szłyk, E., Twarużek, M., Błajet-Kosicka, A. and Grajewski, J. (2017): Determination of mycotoxins, alkaloids, phytochemicals, antioxidants and cytotoxicity in Asiatic ginseng (Ashwagandha, Dong quai, Panax ginseng). *Chemical Papers;* 71(6): 1073–1082.

Gao, S., Duan, X. and Wang, X. (2013): Curcumin attenuates arsenic-induced hepatic injuries and oxidative stress in experimental mice through activation of Nrf2 pathway, promotion of arsenic methylation and urinary excretion. *Food and Chemical Toxicology,* 59: 739–747.

Gwari, G., Bhandari, U., Andola, H., Lohani, H. and Chauhan, N. (2013): Volatile constituents of Saussurea costus cultivated from Uttarakh and Himalayas, *Indian Pharmacological Research,* 5: 179-182.

Ha, H. and Lee, H.B. (2005): Reactive oxygen species amplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney, *Nephrology,* 10(2): 7–10.

Ito, Y., Kobuchi, S., Shimizu, R. and Katsuyama, Y. (2018): Pharmacokinetic and toxicodynamic evaluation of oxaliplatin-induced neuropathy and hematological toxicity in rats. *Cancer Chemotherapy and Pharmacology,* 81:155–161.

Jeong, G.S., Pae, H.O., Jeong, S.O, Kim, Y.C, Kwon T.O., Lee, H.S., Kim, N.S., Park, S.D., Chung, H.T. (2007): The α-methylene-γ-butyrolactone moiety in dehydrocostus lactone is responsible forcytoprotective heme oxygenase-1 expression through activation of the nuclear factor E2-related factor 2 in HepG2 cells. *European Journal Pharmacology,*565: 37-44.

Koek, G.H., Liedorp, P.R. and Bast, A. (2011): The role of oxidative stress in non-alcoholic steatohepatitis. *Clinica Chimica Acta*; 412: 1297-1305.

Kuo, P.L., Ni, W.C., Tsai, E.M. and Hsu, Y.L. (2009): Dehydrocostuslactone disrupts signal transducers and activators of transcription 3 through up-regulation of suppressor of cytokine signaling in breast cancer cells. *Molecular Cancer Therapeutic,* 8: 1328-1339.

Lee, H.K., Song, H.E., Lee, H.B, Kim, C.S., Koketsu, M., Ngan, L.T., Ahn, Y.J. (2014): Growth inhibitory, bactericidal, and morphostructural effects of dehydrocostus lactone from Magnolia sieboldii Leaves on antibiotic-susceptible and -resistant strains of Helicobacter pylori. *PLoS One,* 9: 95530.

Maddux, Betty A., et al. (2001): Protection against oxidative stress—induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α-lipoic acid. *Diabetes,* 50(2): 404-410.

Maria, P.S. and Ronald, B.G. (2006): Lipid management in type 2

diabetes.Clinical *Diabetes.* ; 24(1):27-32.

Mojzisova, G., Mirossay, L., Kucerova, D., Kyselovic, J., Mirossay, A. and Mojzis, J. (2006): Protective effect of selected flavonoids on in vitro daunorubicininduced cardiotoxicity. *Phytotherapy* Research; 20: 110-4.

Nogala-Kalucka, M., Korczak, J., Dratwia, M., Lampart-Szczapa, E., Siger, A. and Buchowski, M. (2005): Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. *Food Chemistry*, 93:227–235.

Parmar, M.P.S., Negi, L.S. and Ramola, S. (2012): Seeds Germination and Seedlings Analysis of Saussurea Costus Royle Ex Benth. In High and Low Altitudinal Villages of District Uttarkashi. *Journal of Pharmacy,* (2) 6: 25-30.

Perandones, C.E., Illera, V.A., Peckham, D., Stunz, L.L. and Ashman, R.F. (1993): Regulation of apoptosis in vitro in mature murine spleen T cells. *The Journal of Immunology,* 151(7): 3521-9.

Ruiz-Larnea, M.B., Leal, A.M., Liza, M., Lacort, M. and Groot, H. (1994): Antioxidant effects of estradiol and 2 hydroxyestradiol on iron induced lipid peroxidation of rat liver microsome. *Steriod;* 59: 383-388.

Ryu, H.S., Kim, J.H. and Kim, H.S. (2007): Effect of Plant Water Extract Mixture (Ixeris sonchifolia Hance (Ish), Oenanthe javanica (Oj), Fagopyrum esculentum Moench (Fem), Hizikia fusiforme (Hf) and Zingiber officinale Roscoe (Zor) on Mouse and the Modulation of Immune Cell Activation. *The FASEB journal,* 21 (5): A375-A375.

Saleem, T.S., Lokanath, N., Prasanthi, A.M., Madhavi, M., Mallika, G. and Vishnu, M.N. (2013): Aqueous extract of Saussurea lappa root ameliorate oxidative myocardial injury induced by isoproterenol in rats. *Advanced Pharmaceutical Technology & Research*, 4(2): 94–100.

Schwingel, T.E., Klein, C.P., Nicoletti, N.F., Dora, C.L., Hadrich, G., Bica, C.G. Lopes, T.G, Silva, V.D. and Morrone, F.B. (2014): Effects of the compound sresveratrol, rutin, quercetin and quercetin nanoemulsion on oxaliplatin-induced hepatotoxicity and neurotoxicity in mice. Naunyn-Schmiedeberg'sArch. *Pharmacology,* 387(9): 837-848.

Seo, M.S. and Choi, E.M. (2012): The effects of dehydrocostus lactone on osteoblastic MC3T3-E1 cells in redox changes and PI3K/Akt/CREB. *Immunopharmacology Immunotoxicolology,* 34: 810-814.

Sethiya, N.K., Trivedi, A. and Mishra, S. (2014): The total antioxidant content and radical scavenging investigation on 17 phytochemicals from dietary plant sources used globally as functional food. *Biomedicine & Preventive Nutrition*, 4: 439–444.

Singh, R., Chahal, K.K. and Singla, N. (2017): Chemical composition and pharmacological activities of Saussurea lappa: A review.

Journal of Pharmacognosy and Phytochemistry, 6 (4): 1298-1308

Steel, R.G. and Torrie, G.H. (1980): Principles and Procedures of Statistics: A Biometrical Approach. New York: McGraw- Hill. p 633.

Sutar, N., Garai, R., Sharma, U.S., Singh, N., Roy, S.D. (2011): Antiulcerogenic activity of Saussurea lappa root. *International Journal* of *Life science and Pharmaceutical Research*, 2: 516-520.

Tag, H.M., Khaled, H.E., Ismail, H.A.A. and El-Shenawy, N.S. (2016): Evaluation of anti-inflammatory potential of the ethanolic extract of the Saussurea lappa root (costus) on adjuvant-induced monoarthritis in rats. *Journal of Basic* and *Clinical Physiology* and *Pharmacology*; 27(1): 71–78

Tao, Z., Raffel, R.A., Souid, A.K. and Goodisman, J. (2009): Kinetic studies on enzyme-catalyzed reactions: Oxidation of glucose, decomposition of hydrogen peroxide and their combination, *Biophysical Journal*, 96 (7): 2977–2988.

Tchamgoue, A.D., Tchokouaha, L.R.Y., Tarkang, P.A., Kuiate, J.R. and Agbor, G.A. (1996): Costus afer possesses carbohydrate hydrolyzing enzymes inhibitory activity and antioxidant capacity in vitro. *Evidence-Based Complementary and Alternative Medicine,2015*; 2015: 10.

Upadhyay OP, Singh RH, Dutta SK. Studies on antidiabetic medicinal plants used in Indian folklore. *Aryavaidyan,* 9: 159-167

Van Wetering, S., van Buul, J.D., Quik, S., Mul, F.P., Anthony, E.C., ten Klooster, J.P., Collard, J.G. and Hordijk, P.L. (2002): Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. *Journal* of *Cell Science*, 115: 1837-1846.

Varga, Z.V., Ferdinandy, P., Liaudet, L. and Pacher, P. (2015): Drug-induced mitochondrial dysfunction and cardiotoxicity. *American Journal of Physiology-Heart and Circulatory Physiology;* 309: 1453-67

Vincent, D.T., Ibrahim, Y.F., Espey, M.G. and Suzuki, Y.J. (2013): The role of antioxidants in the era of cardio-oncology. *Cancer Chemotherapy* and *Pharmacology*, 72: 1157-68.

Zahara, K., Tabassum, S., Sabir, S., Arshad, M., Qureshi, R. and Shoaib, A.M. (2014): A review of therapeutic potential of Saussurea lappa-An endangered plant from Himalaya Asian Pac. *Journal* of *Tropical Medicine*, 7(1): 60-69.