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Article

Comparative *in vivo* study on the role of aqueous and ethanol extracts *of Moringa oleifera* leaves in mitigating cyclophosphamide-induced cardiac toxicity

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ABSTRACT: Cyclophosphamide (CYP) is one of the most invasive common chemotherapeutic anticancers drugs had abroad spectrum of antitumor activity in treatment of human cancer. Its duration and dose produce many side effects. One of the important side effects of CYP is the cardiac toxicity. This study evaluated the effect of *two Morniga oleifera* leave extracts (MLEs): ethanolic leaves extract (ELE) and aqueous leaves extract (AQLE) on CYP induced cardiac toxicity. Forty eight males albino rats divided into six groups based on the received feed dose during in-vivo experiment:1) Control group: Saline solution for 15 days orally; 2) CYP group: injected three times with 50 mg CYP/kg through intraperitoneal injection in the days from 13th to 15th; 3) AQLE group: 500 mg AQLE /kg/day orally through an intra-gastric tube for consecutive 15 days; 4) 500 mg ELE/kg/day orally for consecutive 15 days; 5) AQLE+CYP group: 500 mg AQLE /kg/day orally for consecutive 15 days with 50 mg CYP/kg at the 13th, 14th and 15th days after 1 hour from AQLE administration; 6) ELE+CYP group: 500 mg ELE/ kg/day orally for consecutive 15 days with 50 mg CYP /kg on the 13th,14th and 15thday. In CYP-treated group: the relative body weight, hematological parameters, anti-apoptotic marker and antioxidant enzymes significantly decreased; lipid profiles, cardiac enzymes, anti-inflammatory markers, oxidative marker; malonodialdhyde (MDA) and apoptotic marker (caspase-3) significantly increased. The histopathological examination showed injuries in the cardiac tissues. The administration of MLE with CYP ameliorates the markers of the cardiac toxicity: oxidative stress, cardiac enzymes, lipid profiles and anti-apoptotic marker; B-cell leukemia/lymphoma 2 (BCL2) protein. MLEs protected heart structure and functions against CYP-induced injury through antioxidant and anti-apoptotic activities. The ELE was more effective than AQLE.

1. INTRODCTION

CYP is an alkylating immunosuppressive anticancer drug used in combination chemotherapy for conditions such as: Hodgkin's, non-Hodgkin's Burkitt's lymphoma. It exhibits potent anticancer activity against leukemia, lupus erythematosus, rheumatoid arthritis, multiple sclerosis, myeloma, neuroblastoma, breast, endometrial and lung cancers [1-6]. During bioactivation, CYP generates reactive oxygen species (ROS) induce oxidative damage of cancer cells while affecting normal cells [7, 9]. The overproduction of ROS increases oxidative stress. CYP as a nitrogen mustard alkylating agent contains electrophilic alkyl groups binding the nucleophilic protein moieties of DNA [10]. Its metabolism yield phosphoramide mustard, an antineoplastic agent crosslinks with N7 position of guanine and cytidine bases in DNA, inhibiting replication and enhancing apoptosis of cancer cell. However, its toxic metabolite acrolein causes hemorrhagic cystitis, bone

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marrow suppression, cardiotoxicity and gonadal toxicity with cumulative doses increasing carcinogenic risk [10, 11].

Single large CYP dose killed hemorrhagic cell, caused heart failure or death and cardio toxicity with unclear mechanism. The administration of antioxidant with CYP decreased these side effects. Various natural antioxidants from medicinal plants used globally in treatment different ailments for attenuation and protection against cardio toxicity caused by CYP [12-13].

Phytochemical screening discovered significance therapeutically natural valuable medicinal compounds to manage natural wealth [14-15]. *Moringa oleifera* (MO) is one of the 13 species of *Moringa* (*Moringaceae*) abundant at Sub-Himalayas and now being cultivated worldwide due to the unlimited benefits [14]. It called miracle vegetable because of its medicinal and nutritional value. Leaves, flowers, seedpods, seeds, roots, bark and gum are food sources. The bioactive constituents used in traditional treatment of various illnesses and health improvement. Leaves are highly nutritious, rich in vitamins (A, B, C, E), amino acids, β-carotene, minerals, folic acid, riboflavin, pyridoxine, nicotinic acid and phenols. Leaves used in nutrition of human and animal as well as in soil fertilizers. *M. oleifera* leaf extract (MLE) is a potent anti (microbial, inflammatory, cancer, oxidant) and free radicals' scavenger [15].

The moderate dose of MLE is safe, daily dosage limited to equivalent 70 grams. The frequent excessive intake caused side effects and toxicity. Its low acute toxicity is due the relatively high lethal extract dose 50 (LD50) that causes death in 50% of a test population. The present study aimed the investigation of the antioxidant and the chemoprotective effect of aqueous and ethanolic leaves extract of *Moringa oleifera* against CYP-induced cardiac toxicity in the adult male rats.

2. Materials and Methods

The experimental work carried out in the rat's animals' model with the relevant to the institutional, national and international guidelines and legislation. Ethical template and approval number (Au 0421829202) obtained from Faculty of Medicine-Alexandria University-Egypt.

2.1. Plant Material

The leaves of MO collected from *Moringa* trees cultivated in private farm-Al-Shobak AlGarby-Shebin Al-qanater El-khayria, Qaliubiya governorate, Egypt. Leaves collected with the permission of conducting and publishing this research. The transferred immediately to chemistry Lab. Faculty of science-Alexandria University).

2.2. Chemicals

All chemicals, including CYP and reagent kits obtained from Gomhoureya Co. for Drugs and Medical Supplies-Cairo-Egypt. Absolute ethanol and saline solutions purchased from El Gomhouria Co.-Alexandria-Egypt.

2.3. Extraction and characterization of *M. oleifera* leaves extract

2.3.1. Extraction of *M. oleifera* leaves

M. oleifera leaves washed with tap water, spread and shade-dried for six days, then heat dried at 35-40°C in van air circulating oven and finely grounded into powder. For AQLE, leaves powder mixed with boiling water for five min., filtered twice through 2-µm pore Whatman filter paper and stored in brown sterile bottle at 4°C [16]. For ELE, leaves powder saturated with absolute ethanol solvent, stirred magnetically for 1.0 h at 50 rpm, and left overnight in ethanol. The supernatant collected, filtered, and the residual powder recrystallized in triplicates from absolute ethanol. After evaporation under vacuum, the remaining solid extract suspended in double-distilled water, yield dark green extract stored at 4°C till the experimental use.

2.3.2. Qualitative and quantitative analysis of *M. oleifera* leaves extracts

Gas chromatography-mass (GC-MS) analysis of MLE carried out using Shimadzu Japan GC QP2010PLUS (fused column 2010 (0.25 nm diameter× 50 mm length) coated with polymethyl silicon. The sample heated from 80°C (1 min.) to 200° C (20 min.) at rate 5°C min^{-1}.The field ionization detector (FID) operated at 300°C, injection temperature 220°C, 50 mL min^{-1} nitrogen gas flow rate, split ratio 1:75. Eluent emptied into mass spectrometer (1.5 kV, sampling time 0.2 sec.) connected to computerized-fed mass spectra (MS) data bank Hermlez 233 M-Z centrifuge (Germany). Active components identified by matching corresponding retention times (Rt), peak area percentage and MS fragments with those in National Institute of Standards and Technology WILEY8.LIB (NIST) database [17]. The extract sample rapidly injected into injection part of the column that along with the column and detector heated to a temperature at which sample constituents turned into gaseous phase. The injection part and FID kept warmer than column to promote sample vaporization and prevent condensation in FID. The active constituents separated in the column between the carrier inert nitrogen gas mobile phase and the solid stationary phase. High flow rate and thermal conductivity of nitrogen accelerated separation with high resolution. The FID response recorded versus (Rt) elapsed between the sample injection and detection. The qualitative automatic detection attained by comparison of the Rt values with published values of known compounds for the appropriate temperature and the stationary phase. The integrated area under peak directly proportional to the amount of the eluted solute [18-20].

2.4. Experimental animal's management

The rats' models were Adult healthy males' albino rats of similar weight range from 175g to 200g acclimatized for two weeks before treatment, housed under standard conditions and provided with a balanced diet and water adlibitum in constant environmental conditions (room temperature with 12h light and 12 h dark cycle). The composition of this commercially available diet formulated to meet adequate nutrition needs for growth and health maintenance. It contained a balanced mixed carbohydrates, proteins, fats, vitamins, and minerals. This specific composition was suitable for rats' species, age, and the specific research goal of this study. The rats kept free access to tap water at all times to be maintained hydrated and healthy [17].

After acclimatization period, the experimental rats' animals divided randomly into six groups, each group had eight animals. Rats received feed for 15 days. Group I (control) rats received 1 mL saline solution orally for consecutive 15 days (untreated check); Group II (CYP): Rats received 1 mL saline solution

orally daily for consecutive 15 days before and after CYP through intra-peritoneal at 50 mg/kg dose injected three times at days 13th, 14th, 15th [21]; Group III (AQLE): received AQLE 500 mg/kg/day orally through an intra-gastric tube for consecutive 15 days; Group IV (ELE): Rats received ELE 500 mg/kg/day through an intra-gastric tube for consecutive 15 days; Group V(AQLE/CYP): Received AQLE 500 mg/kg/day orally for consecutive 15 days before and after CYP injection with dose 50 mg/kg injected three times at days 13th, 14th, 15th; Group VI (ELE/CYP): Received ELE 500 mg/kg/day through an intra-gastric tube for consecutive 15 days before and after CYP injection with dose 50 mg/kg injected three times at days 13th, 14th, 15th. The 500 mg treatment dose/kg body weight selected as an effective safe concentration forth *in vivo* studies [22]. Due to toxicity of 150 g CYP high dose [1], this dose divided into three successive doses. Three days' treatment duration is long enough to exert ideal cardiac damage since the clinical trials reported maximum time of plasma concentration of CYP was 24 h [17].

2.5. Blood Samples collection and rat's dissection

After the last treatment, rats unfed for 24 h and diethyl ether used in anesthetization and then dissected the rats from the ventral body side. Blood collected for serum analysis (stored in small falcon tubes and centrifuged to obtain serum) and collected in EDTA containing tubes for whole blood analysis.

2.6. Tissue preparation

Hearts tissues immediately removed, washed and cut into: The first part used for histological examination and molecular studies. The second part homogenized (10%, w/v) separately, in ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer. Supernatant collected in clean falcon tubes after centrifugation for 30 min at 12000xg at 4ºC and used for further analysis. The following assays carried out to analyze the pharmacological activity against CYP induced toxicity in the rats.

2.7. Biochemical analysis

2.7.1. Whole blood analysis

Whole blood samples analyzed for hemoglobin concentration according to cyan methemoglobin method [23] using human co. Drab kin's reagent (Germany). Total leucocytic count determined using haemocytometer method [24].

2.7.2. Serum analysis

After rats' dissection, blood collected in falcon tube and centrifuged at 4000 rpm for 20 min. at 4ºC, Serum samples analyzed for total cholesterol according to Meiattini method and total serum triglycerides determined according to Fossati&Prencipe method. Low density lipoprotein cholesterol (LDL-C) measured according to the method Assmann, 1984, high density lipoprotein cholesterol (HDL-C) measured according to Burstein method, 1984 [25-28]. Serum Creatine kinase- MB (CK-MB) determined by using commercially available kits (Human diagnostic, Germany), Troponine-i assayed by using the enzyme linked immuno assays (ELISA) kits (Kamiya biomedical company, USA). The precision was less than ten percentage to ensure consistent results over time and between kits.

2.8. Cardiac tissue-homogenate analysis

2.8.1 Inflammatory biomarkers estimation in heart tissues

Cardiac Tumor necrosis factor-alpha (TNF-Alpha) level determined using the ELISA technique using the human TNF-alpha kit purchased from Orgenium Company, Vantaa FINLAND according to Intiso method [29]. Cardiac nuclear factor kappa B (NF-$_K$B) level in heart tissue determined by ELIZA technique using the human NF-$_K$B ELISA kit purchased from Glory Science Co., Ltd, USA.

2.8.2. Lipid peroxidation assay

Lipid peroxidation assayed by measurement of MDA levels produced from the oxidative degradation of polyunsaturated fatty acids. MDA reacts with thiobarbituric acid. The developed color measured at 532 nm, according to the method of Buege and Aust (1987) [30].

2.8.3. Catalase (CAT) activity

CAT **(**EC1. 11. 1. 6) (Aebi, 1984) [31] activity measured using shah *et al.* (2013) methodology which relies on decomposition of H_2O_2. An aliquot 25 mL tissue homogenate added to 100 mL of 10mM H_2O_2 and 625 mL of 5 mM EDTA buffer (pH 8.0). The disappearance of H_2O_2 in the reaction mixture by catalase measured spectrophotometrically at 230 nm. CAT activity expressed at U/g tissue.

2.8.4. Superoxide dismutase (SOD) activity

Measurement of SOD activity was based on methodology of (Nishikimi, 1972) [32]. This assay relies on the enzyme ability to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye.

2.9. Quantitative real time PCR and gene expression (Gene expression more specific than protein expression) for monitoring apoptic and antiapoptic markers

Total RNA extracted from cardiac tissues by using Invitrogen™ TRIzol™ Plus RNA purification Kit (simple reliable rapid isolation of pure total RNA, kit utilizes strong lysis capability of TRIzol™ Reagent, followed by a convenient and time-saving silica cartridge purification protocol from PureLink™ RNA Mini Kit, to purify ultrapure total RNA at 1.0 h. Samples quantified at 260 nm (Bioflux, China). Total RNA used to synthesize single stranded cDNA by using applied biosystems™ High-Capacity cDNA Reverse Transcription Kit which amplified using SYBR Green Master Mix (Thermo Scientific, USA) [33]. Table 1 showed the primers list used for gene amplification. Data analyzed using 2−ΔΔCt method in CFX96 Touch™Real-Time PCR (Bio-Rad Co., USA). Comparative Cycle Threshold (CT) values determined changes in gene intensity& expression and normalized to β-actin (Actb) [34].

2.10. Histological investigations

In histology, heart samples specimens: fixed in 10% formol saline for 24h, washed in tap water, dehydrated by serial dilutions by (methyl, pure ethyl, absolute) alcohol; cleared in xylene and embedded in paraffin wax at 56°C in hot air oven for 24h. Paraffin bees wax tissue blocks prepared for sectioning at 4 microns' thickness by slide microtome. Obtained tissue sections collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination using optical light microscope.

Table 1: Primers used in quantitative real time PCR in rats' tissues

Gene	Gene accession No.	forward (5'->3)	reverse (5'->3)	Product length
Caspase3	NM_012922.2	CTTGGAACGCGAAGAAAAGT	AGCCCATTTCAGGGTAATCC	160
Bcl2	NM_016993.2	ACATCCAATAAAAGCGCTGG	TGCAGGTACCAATAGCACTT	93
β-actin	XM_039089807.1	ATGTGGCTGAGGACTTTGATT	ATCTATGCCGTGGATACTTGG	81

2.11. Statistical data analysis

Data analyzed using IBM SPSS software (version 20.0). The Shapiro-Wilk test used to verify normalization. Quantitative data presented as minimum and maximum range, and mean±standard deviation. The F-ANOVA test used for multi-group comparisons, with post hoc Tukey tests for pairwise comparisons.

3. Results and Discussion

3.1. Characterization of *M. oleifera* leaves extract

The MLE showed many different bioactive phytochemicals at the Rt range from 4.15 min.-43.60 min. (Tables 2, 3). Twenty bioactive components recognized at different Rt. Hexadecanoic acid (11.8%), Benzoic acid, 2,2'(1,2 ethenediyl) bis (10.65%) and di-n-octyl phthalate (8.82%) are the major bioactive components.

3.2. Weight change of rats before and after experiment in the different experimental groups

The effect of CYP on rats' growth of after exposure expressed by weight change in different groups, shown in **Figure 1**. The weight gain of CYP-treated rats significantly inhibited ($p < 0.05$) when compared to the control group. The body weight of rats significantly increased ($p < 0.05$) in the other experimental groups.

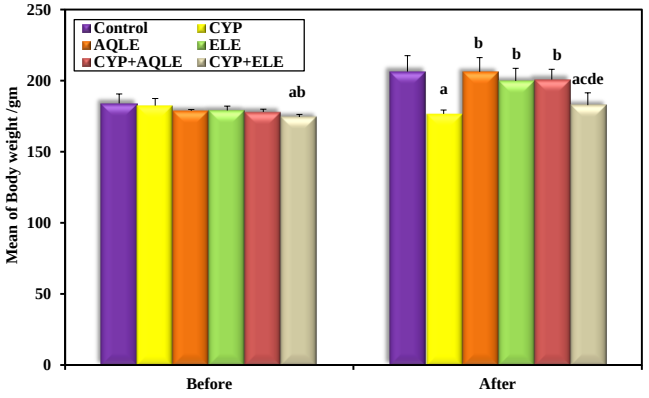

Figure 1. Changes in body weight of CYP treated rats.

The weight gain inhibition in CYP-treated group attributed to immune suppression [62].The active acrolein CYP metabolite produce highly reactive free radicals and affect antioxidant defense system responsible for oxidative damage in different parts and organs of the body that controlled digestion process such as liver secrets bile and some digestion enzymes caused abnormal digestion [63, 64]. This result is consistent with many previous studies confirmed CYP toxicity. Body weight increase in MLEs groups is indicator for MLEs protective role against CYP-induced inhibition of body weight gain.

3.2. Hematological results:

These studies included determination of hemoglobin (Hb) content and total white blood cells (WBCs). The hematological parameters of rats represented in **Figure 2**. CYP administration significantly decreased ($p<0.05$) in Hb content and WBCs number as compared to the control group. The treatment with ELE alone caused a significant increase ($p<0.05$) in Hematological parameter. Treatment with CYP plus MLEs significantly increased Hb concentration and WBCs as compared to CYP-treated group. Pretreatment with ELE was more effective than AQLE.

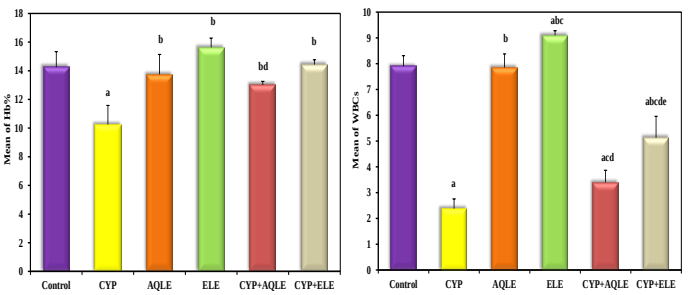

Figure 2. Effect of *MLEs* on hematological parameters of CYP treated rats. CYP was the strongest reducers to Hb and WBCs. ELE is better than AQLE in improving the damaged parameters by CYP.

The reduction of these haematological parameters in CYP-group were due to immunodeficiency disorder that disability of the immune system to defend the body against abnormal and foreign cells which is a major drawback in cancer chemotherapy.These findings in agreement with the result of previous studies which reported that treatment of rat with CYP caused a significant decrease in Hb and WBCs [65] .Chemotherapeutic CYP drug acts on the immune system cells at various levels. Immunosuppression of bone marrow activity as well as innate immune response is the major drawback. Both CYP metabolites, acrolein and phosphoramidemustard responsible for immune suppression [66, 167]. In this study, MLEs applied in cotreatment with CYP significantly increased the protection, Hb content and WBCs number as compared with CYP-treated group. Administration of different MLEs extracts increased WBCs count and Hb concentration. MLEs stimulated the haemopoetic system and immune response, so restored the immune cells production which inhibited by CYP [68, 69].

3.3 Cardiac functional parameters

Cardiac function tests include tests to measure levels of serum Creatine kinase- MB (CK-MB) and troponin-I (cTnI) as circulatory cardiac biomarkers. The protection of MLEs on CP-induced changes in serum CK-MB and cTnI in rats shown in **Figure 3**.

Table 2: Bioactive chemicals in ethanolic leaves extracts.

Compound name	Rt	%Area	Mw. $gmol^{-1}$	Molecular formula	Bioactivity
Benzoic acid,2,2'(1,2ethenediyl) bis	4.2	10.7	268	$C_{16}H_{12}O_4$	OH-isoflavone; improved (metabolism, bone, conservation, apoptosis resistance [35]
Dodecanoic acid methyl ester	21.3	2.6	214	$C_{13}H_{26}O_2$	Antioxidant [36, 37]
Pentadecanoic acid, 14-CH_3, CH_3 ester	28.8	3.4	270	$C_{17}H_{34}O_2$	
Di-n-octyl phthalate	38.5	8.8	390	$C_{24}H_{38}O_4$	
Cyclooctasiloxane, hexadecamethyl	23.8	3.1	592	$C_{16}H_{48}O_8Si_8$	Antibacterial [38]
cis9Hexadecenoic acid	24.8	1.0	298	$C_{16}H_{30}O_2$	Anti-inflammatory, mitigates insulin sensitivity, treating metabolic diseases [39]
9,12-Octadecadienoyl chloride, (Z, Z) (Linoleic acid)	24.8	1.0	298	$C_{18}H_{32}ClO$	Anti-androgenic [40]
Hexadecanoic acid, methyl ester	28.8	3.4	270	$C_{17}H_{34}O_2$	Anti (bacterial, oxidant, fungal, microbial, androgenic flavour), hypocholesterolemic, nematicide, pesticide, androgenic flavor, hemolytic 5-α reductase inhibitor [41, 42]
n-Hexadecanoic acid or palmitic acid	29.7	12.0	256	$C_{16}H_{32}O_2$	hypocholesterolemic, cholesterol solubilizer, anti-oxidant and antitherosclerotic [41, 43]
Hexadecanoic acid, ethyl ester	30.0	2.8	284	$C_{18}H_{36}O_2$	Anti (oxidant, inflammatory, thelmintic, bacterial, allergic, androgenic flavour), hypocholesterolemic, hemolytic, Pesticide, Nematicide, lubricant, 5α-reductase inhibitors [44]
Eicosanoic acid	30		312	$C_{20}H_{40}O_2$	Anti (oxidant, inflammatory) [45]
Octadecanoic acid or stearic acid	30		284	$C_{18}H_{36}O_2$	Anti (inflammatory, oxidant, androgenic cancer preventive, Dermatitigenic-Hypocholesterolemic, 5- α reductase inhibitor [46]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15, 15hexadecamethyl	31.9	6.4	578	$C_{16}H_{50}O7Si_8$	Antimicrobial [47]
Octadecanoic acid, ethyl ester or ethyl stearate	33.3	1.2	312	$C_{20}H_{40}O_2$	
Cyclopentaneundecanoic acid, methyl ester	32.2	1.5	268	$C_{17}H_{32}O_2$	Anti (microbial, oxidant) [48]
Ethyl iso-allocholate	33.0	3.4	436	$C_{26}H_{44}O_5$	Anti (inflammatory, cancer, microbial, antiasthma, and diuretic [49, 50]
Heptasiloxane, hexadecamethyl	39.6	3.1	532	$C_{16}H_{48}O_6Si_7$	Antifungal, anti-inflammatory [51]
DL Arabinose	53.6	0.4	150	$C_5H_{10}O_5$	mitigated high (carbohydrate, fat diet induced metabolic syndrome [52]
D-Lyxose	43.6	0.4	150	$C_5H_{10}O_5$	Precursor in production heart ATP [53]
Lycopene	43.7	0.3	536	$C_{40}H_{56}$	Antioxidant, (heart improving, sun protector, mitigate risk cancer [54]

Table 3: Bioactive chemicals of AQLE

Compound name	Rt	%Area	Mw. $gmol^{-1}$	Molecular formula	Bioactivity
Cyclo-pentane-undecanoic acid, methyl ester	21.32	0.04	268	$C_{17}H_{32}O_2$	Antimicrobial [55]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl	26.58	0.11	578	$C_{16}H_{50}O_7Si_8$	
9,12-Octadecadienoyl chloride, (Z, Z)(Linoleic acid)	24.09	0.06	298	$C_{18}H_{31}ClO$	Anti-androgenic [56]
Methyl tetradecanoate (myristic acid)	25.10	0.53	242	$C_{15}H_{30}O_2$	Anticancer, antioxidant [57]
Hexadecanoic acid, methyl ester (Palmitic acid)	28.80	26.16	270	$C_{17}H_{34}O_2$	Anti (bacterial, fungal, oxidant, inflammatory, hypocholesteroleymic, nematicide, pesticide, 5-α reductase inhibitor [58]
9-Octadecenoic acid methyl ester, (E)	31.87	37.19	296	$C_{19}H_{36}O_2$	Anti (oxidant, viral, cancer) [59]
Pentadecanoic acid, methyl ester (Fatty acid)	35.26	0.54	256	$C_{16}H_{32}O_2$	
Octadecanoic acid, methyl ester	32.25	32.21	298	$C_{19}H_{38}O_2$	Inhibited cardiac arrest-induced neuroinflammation. mitochondrial dysfunction [60]
Methyl stearate	32.25	32.21	298	$C_{19}H_{38}O_2$	Anti (fungal, oxidant) [61]

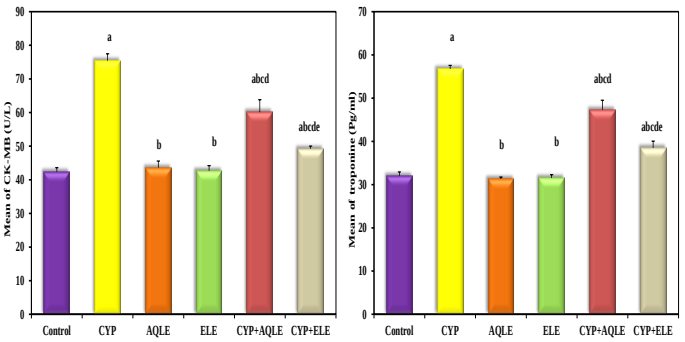

Figure 3. Effect of *MLEs* on cardiac enzymes of CYP treated rats. (CYP increased activity of troponin-I (cTnI) and CK-MB activity and CTnI by increasing sensitive specific biomarkers of myocardial defect. ELE was better than AQLE in mitigation effect of CYP.

The significant increase in CK-MB activity and cTnI Level in CYP-treated group which are high specific and sensitive biomarkers of cardiac defects comparing to the control group was in agreement with Previous study [72].The raised serum CK-MB and cTnI attributed to the increase in free radicals species and their effects on cellular membrane [70] producing leakage of CK-MB and cTnI from the damaged membranes of cardiomyocytes into blood circulation [71], CYP considered as a direct cardiotoxic agent causing endothelial damage and destruction of cardiomyocytes and release of cardiac enzymes into the blood stream [72].MLEs decreased the released cardiotoxicity biomarkers. This result indicating that MLEs have antioxidant properties and the ability to maintain the integrity of heart structure. The protection effect was more marked in ELE group. These results confirmed through the histopathological results, CYP induce hemorrhage, disruption of the muscle structure of the heart and apoptosis, these lesions nearly diminished in MLE+CYP treated groups.

3.4 Lipid and lipoprotein profiles

Data showed a significant increase ($p<0.05$) in TC concentrations as well as the level of TG in CYP-treated rats compared to control group. In CYP + MLE-treated rats, there was a significant decrease in TC and TG concentrations, compared to CYP-treated rats. A significant increase in the levels of LDL-C in CYP-treated rats compared to control group; however, these levels significantly decreased in the CYP+MLE-treated rats. CYP caused a significant decrease in HDL levels compared to the control animals and there was a significant increase in HDL-C in CYP+MLE- treated rats. MLEs-pretreatment to rats treated with CYP showed a significant reduction in serum lipid levels caused by CYP. The ELE pretreatment was more effective than AQLE pretreatment, **Figure 4**.

In present study CYP administration caused abnormalities in lipid levels which indicated CYP toxicity. This is evidenced by a fall in HDL-C level with increased TC, TG and LDL-C levels in rats. Administration MLEs restored the lipid profile alteration. The group administrated with both CYP and MLE showed a significant increase in HDL as well as decrease in TC, TG and LDL depicting less toxicity when compared with CYP group. Elevated concentration of lipid predicted cardiovascular disorder [73]. Cholesterol enhances integrity and stability of the

cardiovascular membrane. The alteration in lipid profile depicted a compromise of membrane integrity and function. The elevation of TG and TC levels in the CYP-treated group indicated that CYP interfering with metabolism and lipids biosynthesis; impaired secretion of heart lipoprotein lipase enzyme (LPL) which responsible for TG degradation to fatty acid, so elevated both TC and TG levels [74]. MLEs hypolipidemic effects attributed to HDL high level, HDL hasten cholesterol removal from peripheral tissues to liver tissues for the catabolism process then excretion and hence labeled good cholesterol [75]. The high level of HDl compete with LDL receptor sites on arterial smooth muscle cells and inhibited LDL uptake and cause degradation of LDL in groups treated with combined CYP and MLEs.

MLEs contains different compounds with antimetabolic syndrome, anti-atherosclerotic activities and anticardiovascular risk factor which acting through lowering levels of cholesterol and triglyceride because they contain 5-alpha reductase inhibitor responsible for HMG-CoA reductase blocking that is a major enzyme in the pathway of cholesterol biosynthesis as mentioned before by Gapalakrishnan and Vadivel 2011[76] in addition to presence of high numbers of bioactive components in MLEs especially ELE which have antioxidant activities and had essential role in hypolipidemic effect of MLEs [77].

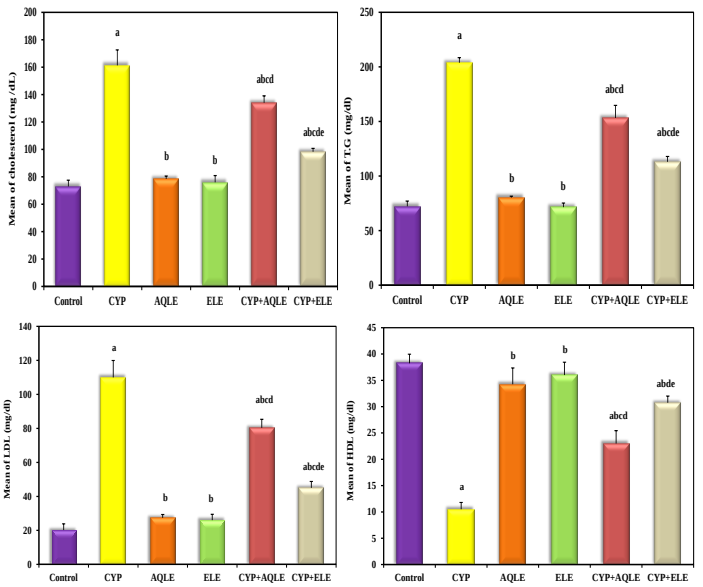

Figure 4. Effect of MLEs on lipids and lipoproteins (CYP increased concentrations of serum lipid, lipoprotein contents and TC and decreased level of HDL. ELE better than AQLE in decreasing all CYP side effects.

3.5. Lipid peroxidation, antioxidant non-enzymatic and enzymatic estimation in cardiac tissue

Protective effect of MLE evaluated *via* characterization the change in the oxidant and antioxidant enzymes by CYP administrated. Lipid peroxidation and antioxidant contents shown in **Figure 5**. The level of the marker lipid peroxidation (MDA) increased compared to the control and the levels of SOD and CAT decreased. MLEs+CYP highly recovered MDA level compared to CYP and increased antioxidants. The ELE was more efficient than AQLE. CYP administration affects the heart mitochondria ability to retain accumulated calcium whose leakage from sarcoplasmic reticulum causing accumulation of mitochondrial calcium, leading to the decreased ATP production and increased ROS releasing [78]. Lipid peroxidation harms cell membrane integrity and affect its functionality, which initiate apoptosis in CYP group and that decreased antioxidant activity [79] indicated by depleted SOD and CAT activities.

CYP causes cardiac damage through lipid peroxidation and redox impairment. As the heart lacks the antioxidant enzymes for scavenging free radicals at the imbalance of ROS generation and elimination [80]. MLE administration reduced lipid peroxidation and increased antioxidants, protected cardiac cells membranes against CYP-induced lipid peroxidation and attenuates the cardiac damage. MLE pre-administration increased SOD and CAT levels in cardiac tissues, preventing or reserving CYP effect of. The total antioxidant activity of MLE attributed to its abundant natural antioxidants content which act as direct antioxidants. Antioxidant properties of *Moringa* leaves confirmed through our GCMs analysis of both extracts of *Moringa* leaves. The large number of components with antioxidant activities as dodecanoic acid methyl ester $C_{13}H_{26}O_2$, hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$, Pentadecanoic acid, 14-methyl, methyl ester $C_{17}H_{34}O_2$, methyl tetradecanoate (Myristic acid) $C_{15}H_{30}O_2$, 9-Octadecenoic acid methyl ester, (E)-$C_{19}H_{36}O_2$ act as antioxidant by inhibiting activation of NADPH oxidase and P47 phox expression.

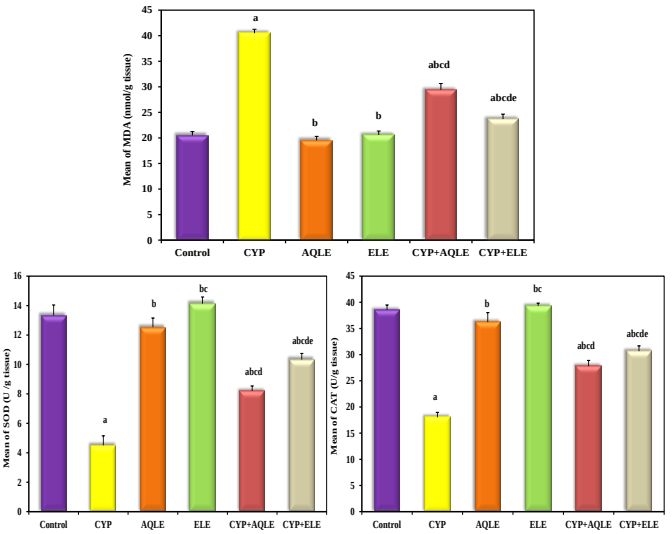

Figure 5. Effect of *M. oleifera* leaves extracts on lipid peroxidation and antioxidants in cardiac tissues CYP increase MDA, while it decreased both SOD and CAT. In comparison to AQLE, the ELE was superior in mitigation these side effects of CYP.

3.6. Caspase-3 and Bcl2 in cardiac tissues

Figure 6 showed changes expressions caspase-3 and B-cell lymphoma2 gene (BCL2) in cardiac tissues. CYP significantly increased caspase-3 expression and decreased expression Bcl-2 compared to the control. *MLEs* protected against CYP declined

caspase-3 and increased BCL2 compared to CYP. ELE was more effective than AQLE. Oxidative stress and ROS generation regulated apoptosis and exerts both antagonistic and agonistic effects on apoptic signaling [81]. CYP enhanced apoptosis by increasing oxidative stress causes pro apoptic proteins translocation to outer mitochondrial membrane (increased permeability) [82]. Cytochrome c which released into cytosol activates downstream effector caspases. Bcl-2 stabilizes mitochondrial membrane and preserving its integrity *via* inhibition cytochrome c release. Apoptosis confirmed by high caspase-3 level caused by CYP while decreased anti-apoptotic protein Bcl-2 as reported [83]. Apoptosis inhibited by MLEs (antiapoptotic contributes prevention of cardiac tissue damage. Antiapoptotic potential confirmed by bioactive components with antiapoptic and anticancer activity in both MLEs. Many phytocomponents have anticancer and antiapoptotic activities such as benzoic acid,2,2'(1,2 ethenediyl) bis,9-Octadecenoic acid methyl ester, (E) Ethyl iso-allocholate and Octadecanoic acid or stearic acid.

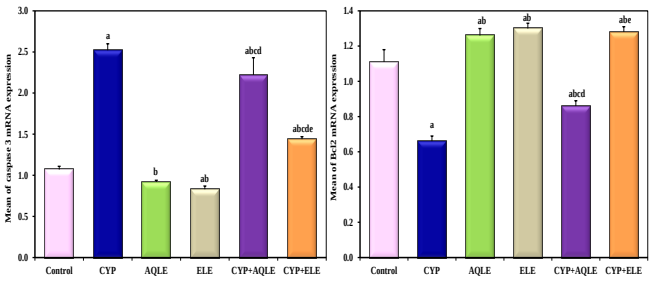

Figure 6. Effect of *M. oleifera* leaves extracts on expression of caspase 3 mRNA and cardiac BCl2 mRNA.

3.7. Inflammatory response markers

Figure 7 showed that a significant increase in cardiac levels of TNF-α and NF-kB as cardiac inflammatory biomarkers in CYP group as compared to normal control group due to ROS-derived oxidative stress increase the expression of NF-kB that is a key mediator of the inflammatory response which plays essential role in cardiotoxicity induced by CYP. NF-kB is an important molecular target for the anti-inflammatory chemicals for cytoprotection against CYP [84]. Inhibition of NF-kB/TNF-pathway prevented tissues toxicity induced by CYP including heart tissues [85].

Pretreated with MlE countered NF-kB activation and reduce inflammation and resulted in significantly decline in cardiac TNF-alpha, this reduction indicated that MLE has anti-inflammatory properties which protected the heart against inflammatory response induced by CYP [86]. MLEs contain many phytochemical components with anti-inflammatory bioactivity as hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$, cis-9-Hexadecenoic acid $C_{16}H_{30}O_2$, Hexadecanoic acid, ethyl ester $C_{18}H_{36}O_2$, Eicosanoic acid $C_{20}H_{40}O_2$, stearic acid $C_{18}H_{36}O_2$ Ethyl iso-allocholate $C_{26}H_{44}O_5$ heptasiloxane, hexadecamethyl $C_{16}H_{48}O_6Si_7$ in ELE and Palmitic acid, methylester $C_{17}H_{34}O_2$, Octadecanoic acid methyl ester $C_{19}H_{38}O_2$ in AQLE. The LE more effective than AQLE due to the more abundant anti-inflammatory. The anti-inflammatory mechanisms for its activity suggested by decreasing oxidative stress in the inflammation condition, inhibition of cyclo-oxygenase enzyme which inhibited prostaglandin synthesis mediated through prostaglandin pathway and obstructive production of several cytokines including TNF-alpha by those phytoconstituents [87].

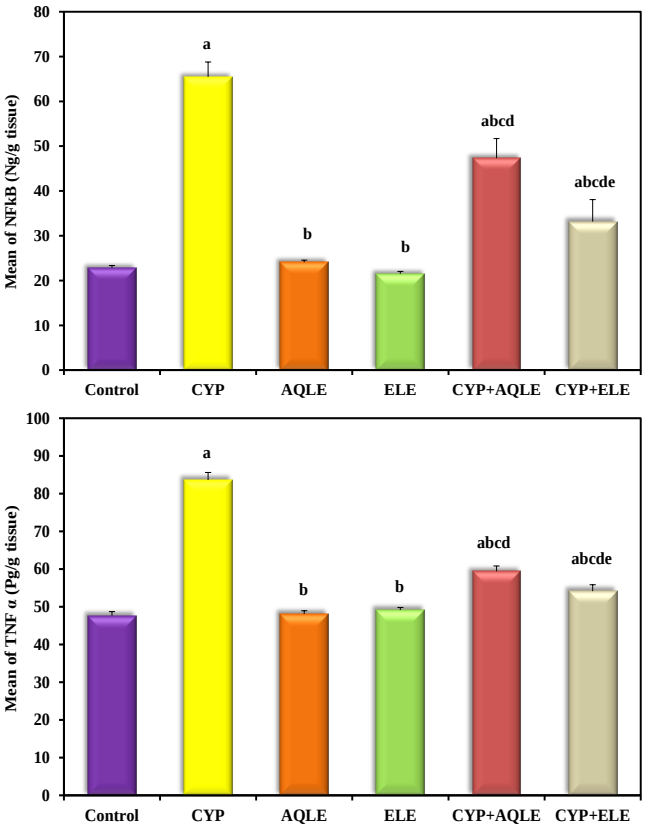

Figure 7. The effect of MLEs and CYP on NFk-B and TNF α levels in different experimental groups of albino rats; CYP:, AQLE: aqueous leaf extract, ELE: ethanolic leaf extract, CYP+AQLE : Co-treated CYP/ AQLE, CYP+ELE : Co-treated CYP-ELE.

3.8. Histopathological findings in cardiac tissues

Light micrographs images of paraffin sections stained with haematoxylin and eosin for morphological changes in rats' cardiac tissues in normal and experimental treatment. The histopathological changes illustrated in Figure 8. Microscopically, the cardiac tissues of the control group showed normal histological picture (Figure 8a). However, section of rat heart tissue from CYP-groups showed marked disorganized myocardium bundle (MB) beside some myocardium cells have dark nuclei (M) with compact cytoplasm and marked edema (E) between myofibrile and bundle, marked dilated and congestive blood vessels (BV), few pyknotic cells (P) seen (Figure 8b). Photomicrograph of rat heart tissues of AQLE+CYP group showed area of discharacterize myocardial bundles (MB), few pyknotic cells (P), mild edema between myocardium cells (E) and congested blood vessels (BV) seen (Figure 8c). Micrograph of ELE+CYP rat hearts showed area of irregular bundle (IB),

some regenerative myocardium cells have dark nuclei (M) with compact cytoplasm, mild dilated and congestive blood vessels (BV) and few pyknotic cells (P) seen (**Figure 8d**). The result illustrated a good correlation between cellular damage and cardiac enzymes levels (CK-MB and CTnI) increased in CYP group and the enzymes levels restored after MLEs treatment .Histopathological evaluation supported the concept that MLEs treatment had a protective effect on cardiac morphology. ELE has high efficacy than AQLE, as there was no edema in CYP+ELE group. The CTnI increased in CYP group and the enzymes levels restored after MLEs treatment. Histopathological evaluation supported the concept that MLEs treatment protective the morphology. ELE has high efficacy than AQLE, as there was no edema in CYP+ELE group.

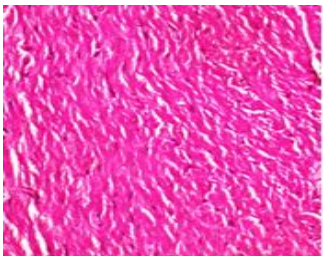

Figure 8a (H& E X400)

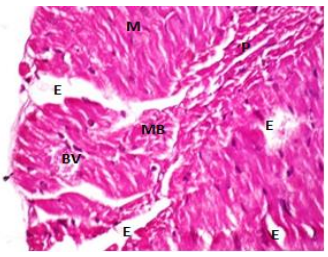

Figure 8b (H& E X400)

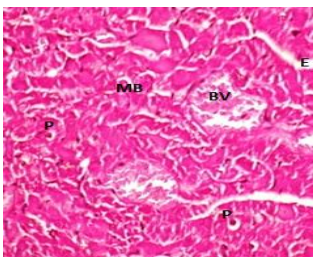

Figure 8c (H& E X400)

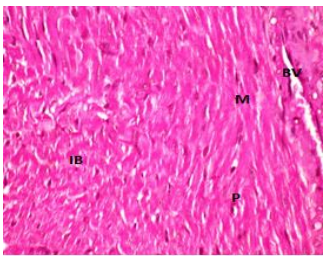

Figure 8d (H& E X400)

Figure 8. CYP-injected rats hearts sections (with/without MLEs :(a) Control rats; (b) CYP-injected rats; (c) CYP + aqueous leaf extract (AQLE) of *moringa*; (d) CYP + ethanolic leaf extract (ELE) of *moringa*.

4. Conclusion

The ethanol and aqueous leaves extract of *M. oleifera* mitigated cardiac toxicity in rats induced by antitumor therapeutic CYP drug. MLEs protected cardiac tissue by abundant antioxidant and anti-inflammatory bioactivities phytochemicals. The free radical scavenging power of MLEs prevented oxidative stress in the cardiac tissues. The *in vivo* tests confirmed that *M. oleifera* extract improved the clinical chemical parameters. The ethanol leave extract was more efficient (in amelioration cardiac enzymes, hematological parameters, lipid profiles, antioxidants and anti-apoptotic markers as well improved Histopathology of the cardiac tissue) by the large numbers of bioactive components. The potent component only in ELE such as DL arabinose which protected against high-carbohydrate, D-Lyxose which present mainly in the heart muscle as a metabolic substrate for production ATP which is important to the health tissues and Lycopene is a powerful antioxidant phytochemical. The limitations are that the antioxidant phytochemicals bioactive anti-inflammatory required cold storage conditions. The stability of these bioactive phytochemical need addressing in a future study for isolation pure bioactive components.

Availability of Data and Materials:

All data generated or analyzed in this study included in this article.

Ethics Approval and Consent to Participate

This study conducted in accordance with ethical guidelines, with approval from Alexandria University's IACUC.

Consent for Publication

All authors consented to publication.

Competing Interests

The authors declare no competing interests.

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