Research Article

Chia Seeds Oil Inhibits Hepatic Resistance to Doxorubicin Via Suppressing Cyp3-A4 and Mrp-1 in Male Albino Rats

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Abstract | ***Background***: The oil-seed of chia (*Salvia hispanica* L.), CSO, had been reported for many biological activities. Doxorubicin (DOX) is one of the active chemotherapies for hepatocellular carcinoma. DOX-resistance is the cause of its limited use. **Objectives**: This study aimed to explore the influence of CSO on DOX-induced resistance and antioxidant status. **Methods**: 40 male albino rats weighing 150-200g were divided equally into four groups 10/each. CSO (5% w/w) were administrated oral daily for 30 consecutive days. DOX treated groups were injected IP at a dose of 2.5 mg/kg trice weekly to be totally 15 mg/kg for two weeks. G1 Control, G2 CSO, G3 Doxorubicin, G4 CSO/DOX. Antioxidant activity, histopathological examination, immunohistochemical analysis of CYP3A4, MRP-1, and c-MYC proteins and expression of miRNAs were assayed. **Results**: Findings indicated that CSO treatment led to a suppression in hepatotoxicity, nephrotoxicity and cardiotoxicity induced by DOX in male albino rats evidenced by a significant enhancement in the activity of ALT, GGT, LDH, AST, also creatinine and uric acid. CSO induced antioxidant activity GSH, CAT and inhibited MDA. Histopathological examination in sections of liver, kidney and heart tissues were confirmed these findings. In liver tissues, DOX resulted in an observable induction in CYP3A4 and MRP-1, while CSO/DOX showed an inhibition in both proteins. c-MYC was dramatically decreased in DOX group, while CSO/DOX group restored cellular c-MYC. CSO/DOX group resulted in a remarkable inhibition in the tumor suppressor miRNA (let-7a) compared to DOX-induced expression. Moreover, miR-122, as negative regulator of DOX resistance, showed a dramatic induction in CSO/DOX group compared to DOX group. **Conclusion**, CSO is capable of effectively inhibit DOX resistance, induced hepatic, nephro and cardiac antioxidant status, inhibit lipid peroxidation, and restore hepatic function.

Keywords | Chia seed oil, Liposomal-doxorubicin, Biochemical indices, Histopathology, Antioxidant, miR-122, miR let-7a, CYP-3A4, MRP1

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INTRODUCTION

Chia seed (*Salvia hispanica L)* in form oil contain an excessive content of fatty acids, especially polyunsaturated fatty acids (PUFA). omega -3 fatty acids, are the common PUFAs of great interest in chia seeds, more than 20% linoleic acid and more than 60% α linolenic acid of its components which are benefical to the health. (Di Marco et al., 2020) Chia seeds used in raw form, blended

with fruit juices or mixed with other cereals and taken as nutritional supplements due to their economical values and their benefits (Kibui et al., 2018).

It has been reported that chia seed contains fat (15-35%), protein (15-25%), dietary fibre (18-35%) ash (4-6%), and carbohydrates (18-31%). Bioactive chemical composition of chia seeds have the beneficial effects like its potent anti-inflammatory, antioxidant, anti-hepatotoxic, antiviral, antibacterial, reduces LDL oxidation, hypotensive, antineoplastic, laxative, analgesic, vasodilator and blood thinner properties as reviewed in (Marcinek and Krejpcio., 2017; Katunzi-Kilewela et al., 2021).

Liver cancer is the third worldwide cause of deaths (Prasanna et al., 2020). Doxorubicin (DOX) was one of the effective chemotherapy drugs for cancer treatment. DOX was an anthracycline, administered every 21 to 28 days in doses of 60 to 75 mg/m^2 body surface through intravenous route (Prasanna et al., 2020).The common usage of doxorubicin has been limited due to its multiple adverse effects and its resistance, including hepatotoxicity, cardiotoxicity, nephrotoxicity (Renu et al., 2018, 2019a,b). Doxorubicin -induced hepatotoxicity mainly due to the production of reactive oxygen species (ROS) throughout its hepatic metabolism that led to imbalanced redox potential and leads to mitochondrial dysfunction, oxidative stress, inflammation, reduced antioxidant enzymes, and apoptosis. (Prasanna et al., 2020).

Intensive clinical efforts are performed on DOX regimens for hepatocellular carcinoma (HCC) treatment to identify new formulas for its delivery or to find combining agents that enhance its activity and reduce its resistance at lower doses to reduce its adverse effects. Therfore, Our study evaluated the therapeutic impact of CSO treatment on DOX-induced toxicity, histopathological examination of liver, kidney and heart tissues, the effect of combination of CSO with DOX on the doxorubicin-metabolizing enzyme Cytochrome p450 3A4 (CYP-3A4) and on the Multi-drug Resistance Protein 1 (MRP-1), evaluation of c-MYC by immunofluorescent cytochemical analysis and expression of miRNAs in liver tissues in male albino rats.

MATERIALS AND METHODS

Materials

Liposomal Encapsulated Doxorubicin; 300115S-IEA, Sigma-Alderich, USA) was used in this study with/ without the cold pressed chia seed oil (CSO; Nature in Bottle, NY, USA). Where the producing company documents its composition (6.0-8.0% Palmitic acid; 0.5% Palmitoleic acid; 3.0-4.5% Stearic acid; 6.0-9.0% oleic acid; 17.0-22.0% linoleic acid; 58-65% alpha linolenic acid; and 0.5% arachidic acid)

Experimental design

Forty male albino rats weighing 150-200g were divided equally into four groups 10/each. CSO 5% (w/w) were administrated oral daily for 30 consecutive days. After 14 days. The treated groups with DOX were injected intraperitoneally 2.5 mg/kg trice weekly after two hours from administration of CSO for two weeks to be totally 15 mg/kg.**G1.**control, **G2.**CSO, **G3.**DOX, and **G4.**CSO/DOX. After the last dose of treatment, the animals were sacrificed, serum was separated for biochemical analysis as ALT, GGT, creatinine, uric acid, LDH and AST, liver, kidney and heart tissue homogenates were harvested for GSH, CAT and MDA, histopathological examinations were performed in liver, kidney and heart tissues. liver tissues were harvested for immunofluorescence detection of CYP3A4, MRP1, c-MYC and expression of microRNAs

Oxidative Stress biomarkers

Liver, kidney and heart tissue homogenates were used for evaluation of GSH , CAT and lipid Peroxidation as MDA concentrations using commercial bio diagnostic kits.

Serum biochemical analysis

The stored serum was used for evaluation of hepatic, nephro and cardiac injury biomarkers respectively. ALT, GGT, creatinine, uric acid, LDH and AST were assessed using commercial bio diagnostic kits.

Histopathological Examination

Paraffin embedded, liver, kidney and heart issue sections (5-7mm) for histopathological examination. for 24 hours, liver , kidney and heart tissues in different rat groups were fixed in 10% neutral buffered formalin and ascending grades of ethyl alcohol (50-100%(hydration). then cleared using xylene (2 changes), then embedded in melting paraffin wax (56 degree) then blocked and cutted in ordinary microtome (3-7 micrometer) thickness and stained with H&E stain and finally checked and photomicrography. (Smith, 2006)

Immunofluorescent detection of CYP3A4 , MRP1 and c-Myc

Fixed rehydrated liver tissue sections (5-7µm thick) were performed by immersing slides in antigen retrieval solution in a beaker and incubated at 99˚C in water bath for 20 minutes. Afterwards, Slides were directly immersed in pre-cooled antigen retrival solution for 5 min at -4 ċ. In a blocking solution, slides were then incubated at 37˚C for 30 min to prevent non-specific binding of antibody. Anti cytochrome p450-3A4 (CYP-3A4)] EPR21062[(ab124921,Abcam,Germany), anti multi drug resistance-associated protein 1 (MRP1)]EPR21062[(ab

23383, Abcam, Germany) and anti c-Myc]EPR17923) [ab185655) were prepared for dilution, After dilution, 100 µl were added to each slide. then slides were immersed in cold buffer for 2 changes 10 minutes each . Alexa Flour 488 anti-rabbit IgG secondary antibody was used and then after dilution, 100 µl were added to each slide.Fluorescent images were visualized using a fluorescent microscope (Yarilin D et al., 2015).

Expression of miRNAs

Total miRNAs were extracted from the liver tissues for all rat groups by the miRNeasy RNA extraction kit (217004, Qiagen, Germany). Then a miScript RT II kit (218161, Qiagen, using 1 µg of RNA) was used for reverse transcription. a NanoDrop™ 2000 was used for quantification of cDNA. the Stratagene Mx3000p real-time PCR system (Agilent, USA), was used for PCR amplification with the miScript Syber green PCR kit (218073, Qiagen). 3 ng cDNA and miRNA primer for has-mir-122-3p (MIMAT0004590: 5'AACGCCAUUAUCACACUAAAUA) hsa-let-7a-3p (MIMAT0004481: 5'CUAUACAAUCUACUGUCUUUC), and U6 (339306). Relative miRNA expression levels have been calculated using the ΔΔCt method[32] and the values have been normalized to the U6 expression (Livak and Schmittgen, 2001).

Statistical Analyses

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test (Chan, 2003). P-values less than 0.05 were considered as statistically significant

RESULTS

Oxidative stress biomarkers

Effect of CSO on hepatic antioxidant biomarkers and Lipid Peroxidation: Our study revealed that hepatic GSH levels showed a decrease in DOX-treated group 15 mg/kg B.W., while CSO/DOX group showed an increase significantly in GSH levels in comparing with DOX group Fig 1a.Table.1. CAT is a very important enzyme in protecting the cell from oxidative damage. CAT activity showed a decrease in DOX group, While CSO /DOX group showed an increase significantly in its activity in comparing with DOX group Fig 1b. Table 1.DOX group showed an increase in MDA levels, while CSO/DOX group showed a significance decrease in MDA levels as compared with DOX group Fig.1c.Table 1.

Effect of CSO on nephrotic antioxidant biomarkers and Lipid Peroxidation: Our study showed a decrease in renal GSH levels in DOX group, while CSO/DOX group showed an increase significantly in GSH levels as compared with DOX group Fig 2a. Table.2.CAT activity showed a decrease in DOX group, While CSO/DOX group showed a significant increase in its activity in comparing with DOX group Fig 2b.Table.2. MDA levels showed an increase in DOX group, while CSO /DOX treated groups showed a significant decrease in MDA levels in comparing with DOX group Fig. 2c. Table.2.

Table 1: Effect of CSO on liver GSH, CAT and MDA in different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Groups	GSH (mg/g)	Catalase (U/g)	MDA (nmol/g)
Normal	29.67±3.56	28.03±2.79	50±3.63
CSO	33.17±3.06	29.4±2.79	39±2
DOX	14.67±3.2 a*	16.67±1.61 a*	79.33±10.58 a*
CSO+-DOX	24.5±2.26 b*	25.1±2.43 b*	55±4.43 b*

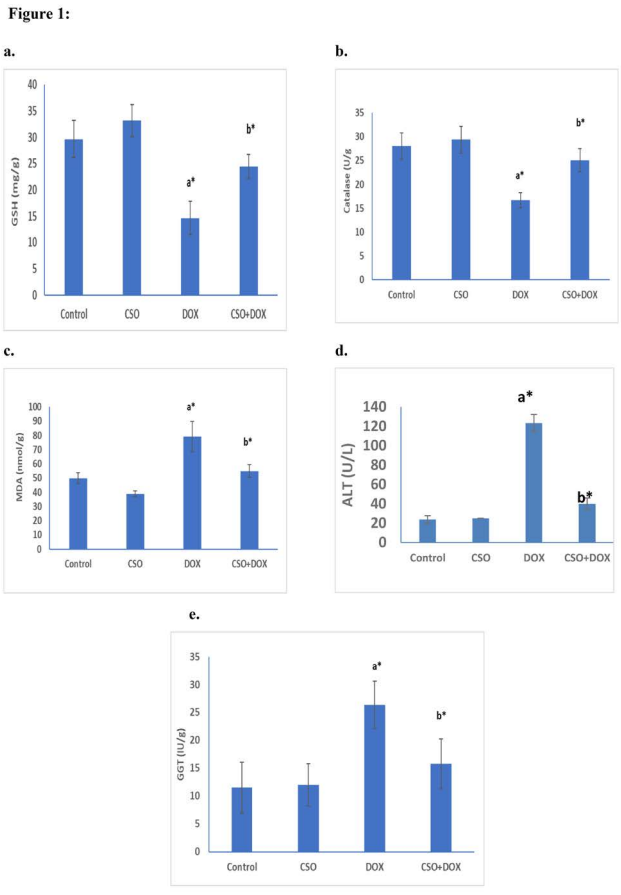

Figure 1: Effect of CSO on liver GSH (a) CAT (b) and MDA (c) concentration in different rat groups. Activity of ALT (d) and Gamma Glutamyl Transferase (e) enzymes in serum of different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05)

Figure 2

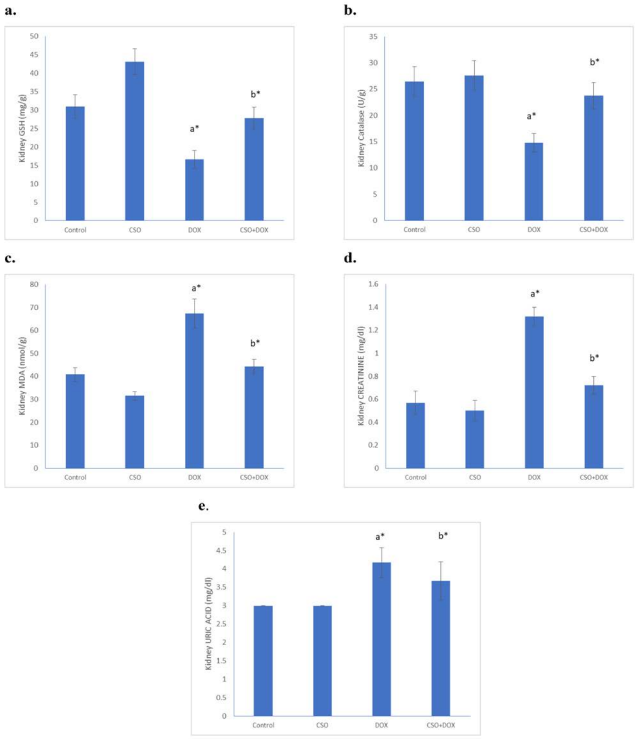

Figure 2: Effect of CSO on kidney GSH (a) CAT (b) and MDA (c) in different rat groups, concentration of creatinine enzyme (d) and uric acid (e) in serum of different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Table 2: Effect of CSO on kidney GSH, CAT and MDA in different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Groups	GSH (mg/g)	Catalase (U/g)	MDA (nmol/g)
Normal	31±3.22	26.47±2.82	40.83±2.99
CSO	43.17±3.54	27.62±2.84	31.5±1.87
DOX	16.67±2.42 a*	14.83±1.75 a*	67.33±6.38 a*
CSO+-DOX	27.83±2.99 b*	23.73±2.53 b*	44.33±3.01 b*

Effect of CSO on cardiac antioxidant biomarkers and Lipid Peroxidation: Our study showed that cardiac GSH levels showed a decrease in DOX group, while CSO / DOX group showed an increase significantly in GSH levels as compared to DOX group Fig 3a.Table 3. DOX group showed a decrease in activity of CAT, While CSO /DOX group showed a significance increase in its activity in comparing with DOX group Fig 3b.Table 3. Our study revealed that DOX group showed an increase significantly in MDA levels, while CSO/DOX group showed a significance decrease in MDA levels in comparing with DOX group Fig 3c. Table 3

Figure 3

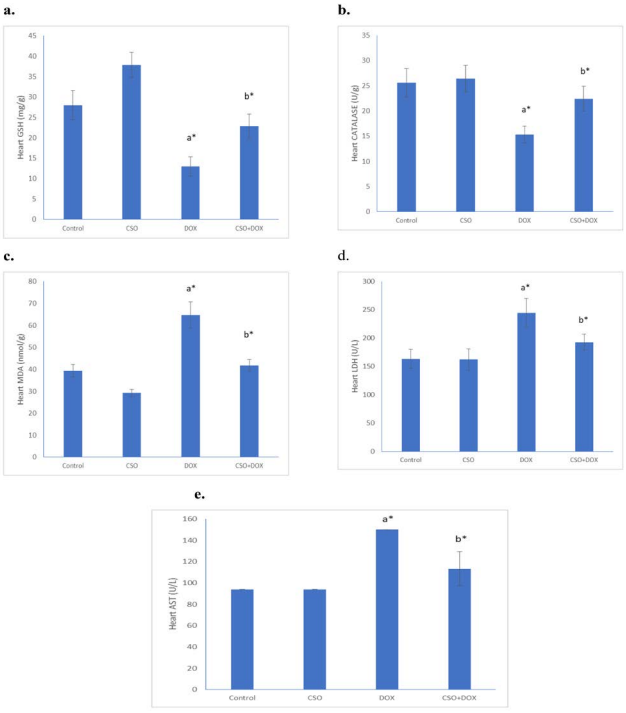

Figure 3: Effect of CSO on heart GSH (a), CAT (b) and MDA in different rat groups, Activity of LDH (d) and AST (e) enzymes in serum of different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Table 3: Effect of CSO on heart GSH, CAT and MDA in different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Groups	GSH (mg/g)	CATALASE (U/g)	MDA (nmol/g)
Normal	28±3.58	25.57±2.79	39.33±2.88
CSO	37.83±3.06	26.4±2.63	29.17±1.72
DOX	13±2.37 a*	15.3±1.7 a*	64.67±6.02 a*
CSO+DOX	22.83±2.99 b*	22.42±2.44 b*	41.83±2.64 b*

Serum biochemical analysis

This study revealed that, DOX group showed a significant increase in ALT activity, while CSO/DOX group showed a significance decrease in ALT activity Fig 1d, Table 4. -Glutamyl transferase levels showed a significant increase in DOX group, while CSO/DOX group showed a decrease in its activity in comparing with DOX group Fig.1e, Table 4. Creatinine concentration showed a significant increase in DOX group, while CSO/DOX group showed a decrease significantly in its concentration Fig.2d, Table 4. Uric acid concentration showed a significant increase in DOX group, while CSO/DOX group showed a significance decrease in its concentration as compared to DOX group Fig.2e, Table 4. The activity of serum LDH showed

Table 4: Effect of CSO on serum ALT, GGT, creatinine, uric acid, LDH and AST in different rat groups. Data are expressed as mean ± SE. Treated Groups showed a significance as compared to [a] control and [b] compared to DOX (*P<0.05).

Groups	ALT (U/L)	Gamma Glytamyl Transferase (u/g)	Creatinine (mg/dl)	Uric Acid (mg/dl)	LDH (U/L)	AST (U/L)
Normal	23.63±3.89	11.5±4.59	0.57±0.1	3±0	163.33±17.1	94±0
CSO	25±0	12±3.79	0.5±0.09	3±0	162±18.93	94±0
DOX	123.25±8.8 a*	26.33±4.27 a*	1.32±0.08 a*	4.17±0.41 a*	244.33±25.51 a*	150±0 a*
CSO+DOX	39.88±6.01 b*	15.83±4.45 b*	0.72±0.08 b*	3.67±0.52 b*	192.67±13.78 b*	113.38±16.04 b*

a significant increase in DOX group, while CSO/DOX group showed a significance decrease in its activity Fig.3d, Table 4. AST activity showed a significant increase in DOX group, while CSO/DOX group showed a decrease significantly in its activity as compared with DOX group Fig.3e, Table 4.

Histopathological Examination of Liver, Kidney and Heart Tissues (H&E)

Paraffin-embedded liver, kidney and heart issue sections (5-7mm). hematoxylin and eosin were used for staining different tissues for histopathological examination to detect the morphological changes. In the liver sections of control rats and CSO-administrated rats showed normal histological structure of central vein, portal area and hepatic cells, as shown in Fig.4. Liver sections of DOX-treated rats showed marked thickening of the hepatic (Glisson's) capsule with edema, few inflammatory cells infiltration, necrosis, degeneration of the subcapsular hepatocytes, diffuse moderate vacuolar necrosis and degenerated hepatic cells with dilated and capillarization of hepatic sinusoids, as shown in Fig.4. Liver sections of CSO/DOX-treated rats showed good restoration of the hepatic cells with only mild degeneration of the hepatic cells, few inflammatory cells infiltrating the portal areas and mild degeneration of the hepatic cells, as shown in Fig 4.

Sections of control and CSO administrated rats showed normal histological structure of renal glomeruli (RG) and real tubules (RT), while DOX group showed mild focal interstitial inflammatory cells infiltration , diffuse moderate tubular epithelial linings degeneration, necrosis, and desquamation with diffuse granular cast formation in the tubular lumens, also showed degeneration, necrosis, few apoptosis and desquamation of the renal tubular linings with cast formation in the tubular lumens and eosinophilic cast formation in the Bowman's space with mild mesangial proliferation. Kidney sections of CSO/DOX group showed mild to moderate degree of necrobiotic changes of the renal tubular epithelium with cast formation in lumen of scattered tubules Fig 5.

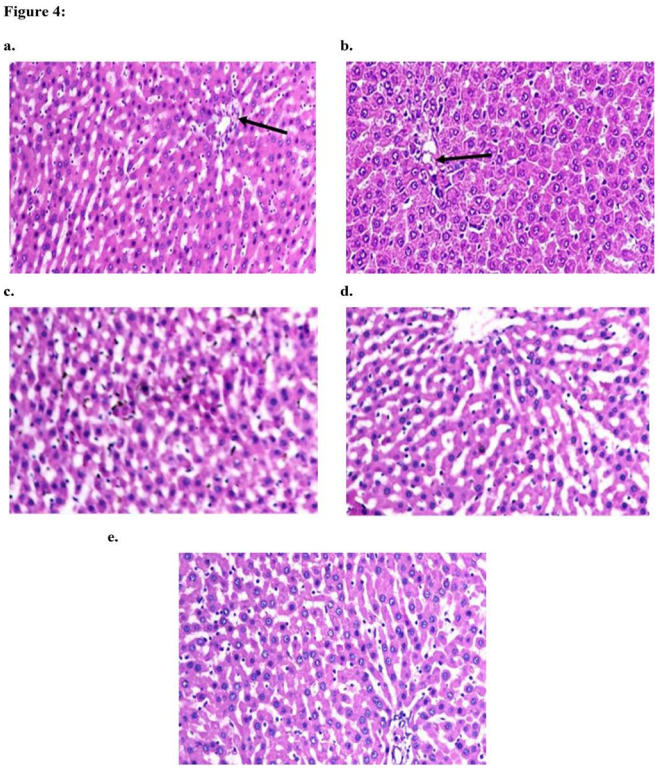

Figure 4: Histopathological examination of rat liver tissues by hematoxylin and eosin: a. control rats and b.CSO-administrated rat are showing normal histological structure in portal area (arrow) and hepatic cells. c. DOX-treated rat showing marked thickening of the hepatic (Glisson's) capsule with edema and few inflammatory cells infiltration, necrosis and degeneration of the subcapsular hepatocytes.d.CSO/DOX-treated rat showing few inflammatory cells infiltrating the portal areas and mild degeneration of the hepatic cells. e. CSO/DOX-treated rat showing good restoration of the hepatic cells with only mild degeneration of the hepatic cells, (X200).

Heart sections of control rats, CSO administrated rats showed normal histological and orientation of the cardiac muscle fibers (MFs) with their centrally located nuclei (arrow), Heart sections of DOX group showed diffuse vacuolar degeneration of the cardiac muscle fibers, most of which showing signet ring appearance (arrow) and necrosis (dashed arrow). intermuscular edema (Ed), mild inflammatory cells infiltration (dashed arrow), vasculitis

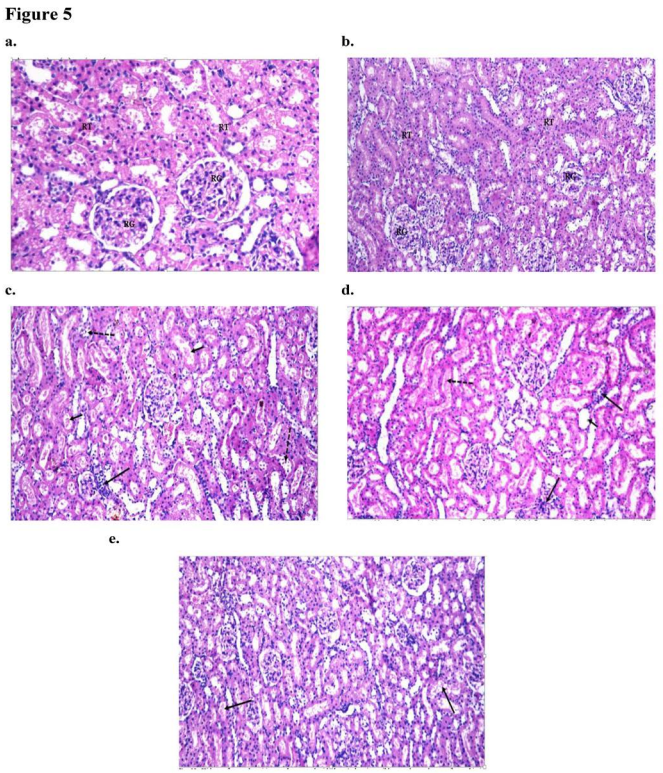

Figure 5: Histopathological examination of rat kidney tissues by hematoxylin and eosin: a. control rats and b. CSO-administrated rat administrated rats are showing normal histological structure of renal glomeruli (RG) and real tubules (RT). c. DOX-treated rats showed mild focal interstitial inflammatory cells infiltration , diffuse moderate tubular epithelial linings degeneration, necrosis d. DOX-treated rat showing degeneration (arrow), necrosis, few apoptosis (dashed arrow) and desquamation (short arrow) of the renal tubular linings with cast formation (thin arrow)in the tubular lumens. e. CSO/DOX-treated rat mild to moderate degree of necrobiotic changes of the renal tubular epithelium with cast formation in lumen of scattered tubules (arrow) (H&E, X100)

(short arrow) and perivascular edema (Ved). Heart sections of CSO/DOX group showed mild vacuolar degeneration (arrow) and very few necrosis of the cardiac muscle fibers and good restoration of the cardiac muscle fibers with only mild vacuolar degeneration (arrow) Fig.6.

Immunohistochemical analysis of CYP-3A4 in liver tissues

Immunohistochemical analysis for CYP-3A4 protein was performed to confirm and the effect of CSO in inhibiting CYP-3A4 concentration. Control liver section showed low CYP-3A4 concentration Fig 7a, while liver section of CSO-treated rats showed low CYP-3A4 concentration Fig 7b. High CYP-3A4 concentration was detected in DOX-treated cells Fig 7c.This CYP-3A4 concentration was inhibited by CSO, as noticed in the stained cells, as shown in Fig. 7d.

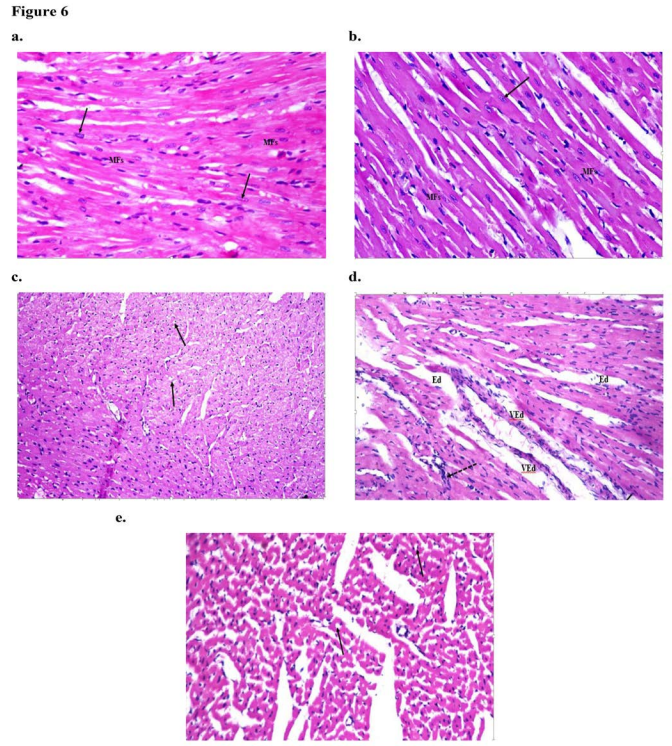

Figure 6: Histopathological examination of rat heart tissues by hematoxylin and eosin: a. control rats and b. CSO-administrated rat showed normal histological and orientation of the cardiac muscle fibers (MFs) with their centrally located nuclei (arrow). c. DOX-treated rats showed showing diffuse vacuolar degeneration of the cardiac muscle fibers, most of which showing signet ring appearance (arrow) and necrosis (dashed arrow), d. Dox-treated rats showing intermuscular edema (Ed), mild inflammatory cells infiltration (dashed arrow) e. CSO/DOX-treated rat showing good restoration of the cardiac muscle fibers with only mild vacuolar degeneration (arrow) and very few necrotic fibers

Immunohistochemical analysis of MRP1 in liver tissues

Immunohistochemical analysis of MRP1 protein was performed to the effect of CSO in inhibiting the concentration of MRP1. The control liver section Fig 8a and the liver section of CSO-treated rats Fig 8b showed low MRP1 concentration. high MRP1 concentration was detected in DOX-treated cells Fig 8c.This MRP1 concentration was depleted by CSO, as observed in the stained cells, as shown in Fig. 8d.

Immunohistochemical analysis of c-MYC in liver tissues

In this study, c-MYC protein was investigated by histochemical analysis to explore the promoting effect of CSO on the known DOX-inhibitory role on c-MYC. The immunohistochemical analysis was performed to confirm these findings. As observed in Fig. 9a, there is a high con

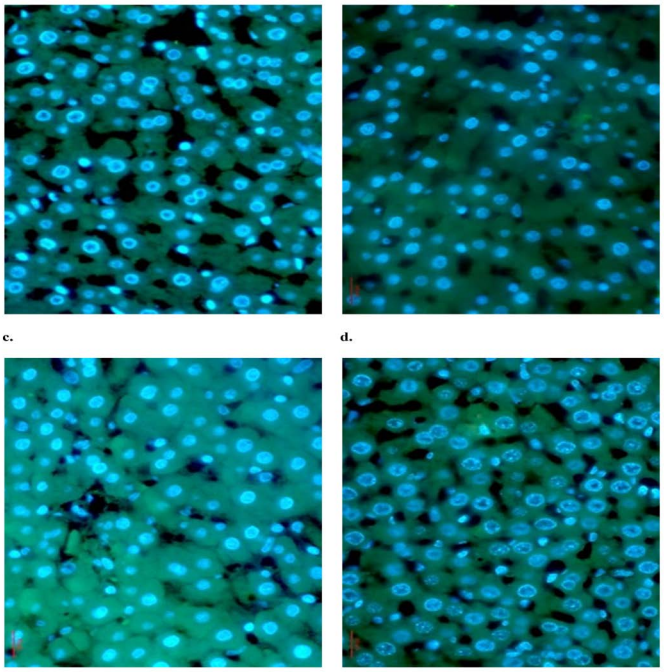

Figure 7: The immunohistochemical staining of CYP-3A4 in rat liver sections was implemented by FITC-conjugated IgG and CYP-3A4 antibody (green) and the nuclei were counterstained with DAPI (blue). Control group (**a**) and CSO-treated group (**b**) showed low concentration of CYP-3A4. Group treated with liposomal-doxorubicin (**c**) showed highly induced CYP-3A4 concentration. CSO/liposomal-doxorubicin group (**d**) showed highly suppressed CYP-3A4 compared to DOX-treated group. The sections were analyzed under fluorescence microscope (Magnification x400).

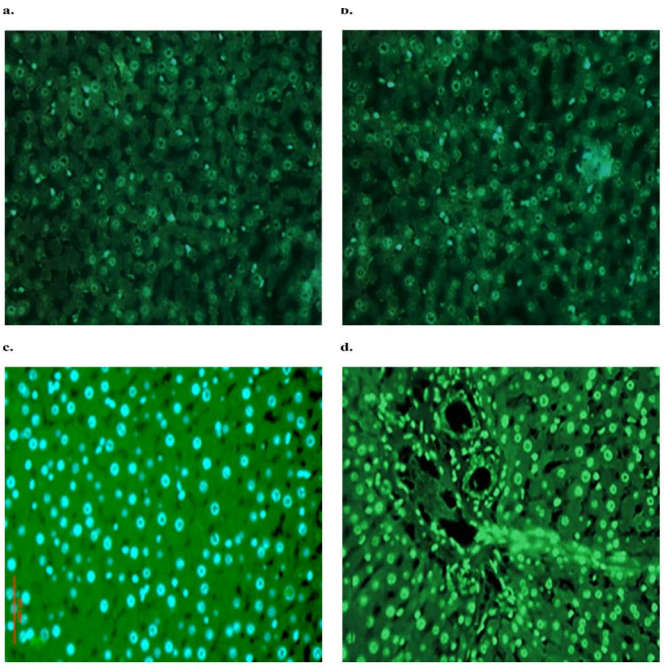

Figure 8: The immunohistochemical staining of MRP1 in rat liver sections was implemented by FITC-conjugated IgG and MRP1 antibody (green) and the nuclei were counterstaining with DAPI (blue). Control group (a) and CSO-treated group (b) showed low MRP1 concentration. The group that was treated with liposomal-doxorubicin (c) demonstrated highly induced MRP1 concentration compared to control group. CSO/liposomal-doxorubicin group (d) showed a highly decreased MRP1, compared to DOX-treated group. The sections were analyzed under fluorescence microscope (Magnification x200).

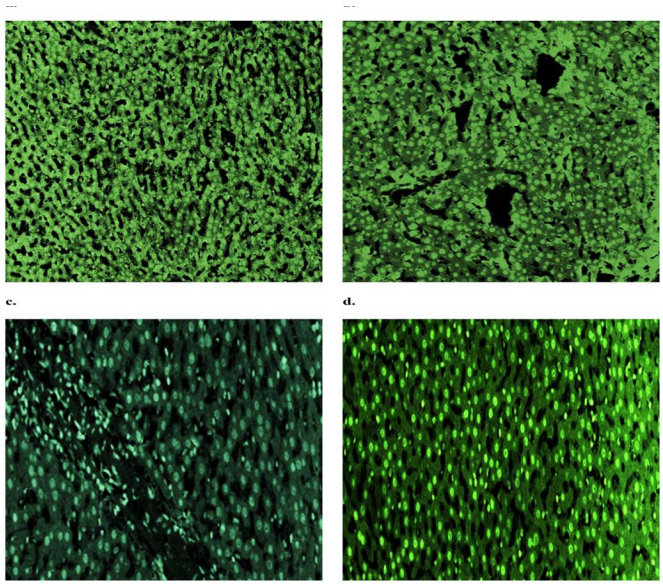

Figure 9: The immunohistochemical staining of c-MYC was implemented by FITC-conjugated IgG and c-MYC antibody (green). Control group (a) and CSO-treated group (b) showed high c-MYC concentration. Liposomal-doxorubicin-treated group (c) indicated low c-MYC concentration compared to control. The analysis of CSO/liposomal-doxorubicin group (d) showed increased c-MYC content than DOX-treated group. The sections were analyzed under fluorescence microscope (Magnification x100).

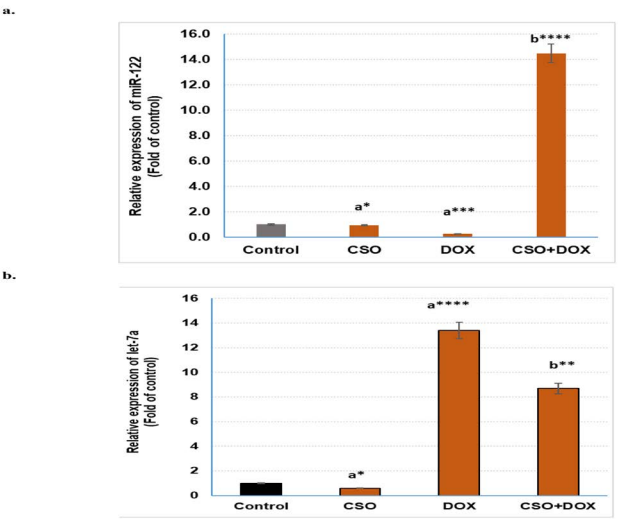

Figure 10: qRT-PCR analysis of the relative expression of miR-122 (a) and let-7a (b). in liver tissues of different groups. Data are expressed as mean ± SE. $^{*}p < 0.05$, $^{**}p <$

0.01, *** p< 0.001, and **** p< 0.0001, [a] compared to the corresponding control, [b] compared to DOX-treated group.

tent of c-MYC in control hepatic tissue, and this status did not change with CSO treatment, Fig. 9b. On the other hand, as obvious in Fig. 9c. Low c-MYC concentration was detected in DOX-treated group. This concentration is restored by CSO, as noticed in the stained cells Fig. 9d

Effect of CSO on the expression of miR-122 and let-7a in liver tissues

The liver tissue was extracted to isolate miRNAs. Among them, miR-122 expression was investigated in different rat groups. The DOX group showed an inhibition significantly in miR-122 (p<0.001), compared to the control group. On contrary, CSO/DOX resulted in a remarkable induction in the tumor suppressor miRNA (miR-122), (p<0.0001) in comparing with DOX group as presented in Fig. 10a. Among the isolate miRNAs from liver tissue, let-7a expression was explored in different rat groups. DOX group showed a dramatic induction in the expression of let-7a (p<0.0001), compared to control group. On contrary, CSO/DOX resulted in a notable inhibition in the tumor suppressor miRNA (let-7a), (p<0.01) compared to DOX group, as presented in Fig. 10b.

DISCUSSION

Doxorubicin is an anthracycline, which can be contributed as a simple agent or in merger with other chemotherapeutic drug in the treatment of cancers, including leukemias, lymphomas, and solid tumors but its administration is limited by a dose-dependent, irreversible, and progressive toxicity (Buzdar AU et al., 1985). There are cytotoxic effects on malignant cells, but are confused by an improvement in the risk of cardiotoxicity, hepatotoxicity, renal insufficiency (Damodar et al., 2014).

Doxorubicin is the most effective cytotoxic agent for HCC treatment. In particular, the drug molecule stabilizes the topoisomerase II complex ;preventing the DNA double helix from being resealed and thereby stopping the process of replication. (Penault et al., 2003).Oxygen-free radicals produced during the metabolic activation of DOX may have toxic effects on heart muscle (Hamlaoui S et al., 2012), which is provided with poor mechanisms of detoxification of such species DOX is likely to have toxic effects on liver (Montgomery MD et al., 2017).

During the treatment of cancer by using anticancer therapeutic agents, most of biochemical and physiological homostsis were destructed. DOX antitumor drug showed wide applications for treating several malignancies, however, its uses are limited by hepatotoxicity, nephrotoxicity and cardiotoxicity development; therefore, much efforts have been focused on the preventative impacts of natural agents against DOX-induced hepatotoxicity, nephrotoxicity and cardiotoxicity (Yagmurca et al., 2003).

In this current in vivo study, GSH in addition to CAT and MDA were evaluated in liver, kidney and heart tissue homogenates. The hepatic levels of GSH and CAT were reduced in rats treated with DOX , while CSO/DOX group exhibited an elevation significantly in hepatic GSH and CAT levels related to DOX-treated group .An elevation in MDA levels was noticed in hepatic tissues in DOX group, while CSO/DOX rat groups showed a significance decrease in hepatic MDA levels. These findings agreed with previous studies demonstrated the reduction of hepatotoxicity induced by DOX (Meng Y et al., 2019).

GSH and CAT renal levels were reduced in DOX group, while CSO/DOX group exhibited an elevation in the renal GSH and CAT levels related to DOX-treated group. renal tissue homogenates showed an elevation in MDA levels in DOX group, while CSO/DOX group showed a reduction significantly in the renal MDA levels in comparing with DOX-treated group. These findings were in agreement with several studies demonstrated the ameliorating effects of natural agents against DOX nephrotoxicity (Abdel Moniem et al., 2014).

GSH besides CAT and MDA were evaluated in the homogenates of cardiac tissues. The cardiac levels of GSH and CAT were reduced in DOX group, while CSO/DOX group revealed an increase significantly in the cardiac GSH and CAT levels related to DOX group. An elevation in MDA levels were noticed in cardiac tissues in DOX group, while CSO/DOX group showed a significance decrease in cardiac MDA levels. These findings were in accordance with several studies demonstrated the ameliorating effects of natural agents against DOX cardiotoxicity (Moussa et al., 2020; Saleh et al., 2020; Hamza et al., 2021).

Our study showed that DOX group induced hepatotoxicity by a significant increase in Serum ALT and GGT levels, while CSO/DOX group caused their levels to be decreased significantly. These findings were in agreement with several studies demonstrated the ameliorating effects of natural agents against DOX hepatotoxicity (MengY et al., 2019). DOX-induced nephrotoxicity was manifested by increasing serum concentrations of creatinine and uric acid in DOX group, while CSO/DOX group showed a significant decrease in their concentrations. These findings agreed were in accordance with several studies demonstrated the ameliorating effects of natural agents against DOX nephrotoxicity (El-Sheikh et al., 2012; Abdel Moneim et al., 2014). LDH and AST levels were increased signifi-

cantly in DOX group induced cardiotoxicity, while CSO/DOX group caused their activities to be decreased. These findings were in agreement with several studies demonstrated the ameliorating effects of natural agents against DOX cardiotoxicity (Alimoradian et al., 2018; Moussa et al., 2020; Saleh et al., 2020).

Histopathological examination for detecting morphological changes in hepatic tissues indicated that CSO-administrated rats showed normal histological structure, while DOX-treated rats showed marked thickening of the hepatic (Glisson's) capsule with edema, few inflammatory cells infiltration, noticed necrosis, degeneration of the subcapsular hepatocytes, diffuse moderate vacuolar necrosis and degenerated hepatic cells with dilated and capillarization of hepatic sinusoids, while CSO/DOX group showed good restoration of the hepatic cells with only mild degeneration, few inflammatory cells infiltrating the portal areas.(Alimoradian et al., 2018; Moussa et al., 2020; Saleh et al., 2020).

Morphological changes in kidney tissues showed that CSO administrated rats showed normal histological structure of renal glomeruli (RG) and real tubules (RT) , while DOX rats group showed mild focal interstitial inflammatory cells infiltration , diffuse moderate tubular epithelial linings degeneration, necrosis, and desquamation with diffuse granular cast formation in the tubular lumens, while Kidney sections of CSO/DOX group showed necrobiotic changes of the renal tubular epithelium with cast formation in lumen of scattered tubules and also showed mild degenerative and necrotic changes of the renal tubular epithelium, eosinophilic cast in some tubular lumens and few congested interstitial blood vessels and good restoration of the renal parenchyma with mild degenerative changes of the renal tubular epithelial linings scarce desquamated cells , and few necrotic cells respectively. These findings were in aggrement with the previous studies that investigated the ameliorating effects of natural agents against DOX nephrotoxicity (El-Sheikh et al., 2012).

Histopathological examination for detecting morphological changes in heart tissues, CSO administrated rats showed normal histological and orientation of the cardiac muscle fibers (MFs) with their centrally located nuclei, while DOX-treated rats showed diffuse vacuolar degeneration of the cardiac muscle fibers, most of which showing signet ring appearance and necrosis. intermuscular edema (Ed), mild inflammatory cells infiltration Heart sections of CSO/DOX group showed mild vacuolar degeneration and very few necrosis of the cardiac muscle fibers and good restoration of the cardiac muscle fibers with their striation and only few degenerated cells respectively. These findings were supported with previous studies demonstrated the ameliorating effects of natural agents against DOX cardiotoxicity (Ozcan.M et al., 2019).

In HCC, three ATP-binding cassette (ABC) subfamilies, ABCB, ABCC and ABCG (BCRP proteins), may be involved in DOX specificities, they have revealed the ability to transport DOX (Ng et al., 2000; Nies et al., 2001). MRP1, MRP2, MRP3 and MDR1 are expressed at the transcriptional level in HCC. MDR1 protein expression occurs in 80-90% of HCC cases (Ng et al., 2000). The family of MDR proteins has also been reported to be expressed and active in mitochondrial membranes, possibly to protect mitochondrial DNA from drug-stimulated damage by maintaining the drug out of the mitochondria or to inhibit apoptosis by modulating mitochondrial outer membrane permeability (Solazzo et al., 2009).

Histochemical analysis of CYP-3A4 and MRP1 in liver tissues indicated that both proteins were low in control and in treated CSO-rats, while both were dramatically elevated in DOX-treated cells. On the other hand, combination of CSO with DOX resulted in an observable inhibition in both proteins. c-MYC has been considered as a key factor in the transcriptional response. c-MYC expression was increased rapidly during the pre-replicative phase that accelerate DNA synthesis process (Zheng et al., 2017).c-MYC is also localized in the nucleus in highly proliferating fetal hepatocytes, that localization of c-MYC indicated that c-MYC is altered in association with cell proliferation, repression or activation of essential target genes that involved in proliferation and growth of liver cell prevented by sequestration that happened in the nucleolus (Sanders et al., 2005). In this current study, the histochemical analysis of c-MYC in liver tissues revealed that protein was high in control and in treated CSO-rats, however c-MYC was dramatically decreased in DOX group, while combination of CSO with DOX restored some of the cellular c-MYC. In this current study, DOX group showed an inhibition in the expression of miR-122 in comparing with control group. On contrary, the combined treatment with CSO/DOX group resulted in a remarkable induction in the tumor suppressor miRNA (miR-122) compared to DOX-treated group. Moreover, as another tumor suppressor miRNA let-7a expression was explored in different rat groups. DOX group showed a dramatic induction in the expression of let-7a, while the combined treatment with CSO/DOX group resulted in a notable inhibition in let-7a compared to DOX group. MiR-122 had been reported to be re-sensitize hepatic carcinoma cells after administration through MDR-inhibiting of its corresponding genes, including MRP and MDR-1. Consequently, in the current study, the upregulated miR-122 after CSO administration may resulted in the elevated sensitivity to DOX through MRP1 suppression.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, The results indicated that CSO showed an improvement significantly in oxidative stress biomarkers by increasing activities of GSH and CAT, decreasing MDA level in liver, kidney and heart tissues. Treatment with CSO led to decrease significantly in ALT, GGT, Creatinine, Uric acid, AST and LDH. Histopathological examinations were performed to confirm these findings. CSO is capable of re-sensitized hepatic cells to doxorubicin effectively via inhibiting MRP1 and CYP-3A4. Moreover, CSO inhibited hepatic resistance to DOX via inducing miR-122 as a negative regulator of DOX resistance and inhibiting let-7a. Additionally, CSO can potentiate DOX cytotoxicity and control the hepatic cell growth via inhibition c-MYC.

LIST OF ABBREVIATIONS

Chia seed oil	CSO
Doxorubicin	DOX
ABC	ATP-binding cassette
CYP-3A4	Cytochrome p450 3A4
MRP1	Multidrug resistance-associated protein
miRNAs	microRNA
PUFA	Polyunsaturated fatty acids
Haemaytoxylin and eosin	H&E
Reduced glutathione	GSH
Catalse	CAT
Malondialdehyde	MDA
Alanine amino transeferase	ALT
Gamma glutamyl transferease	GGT
Aspartate amino transeferease	AST
Lactate dehydrogenase	LDH

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally according to their tasks and approved the final manuscript.

ETHICS APPROVAL

This study was approved by the Ethical Committee of the National Research Centre in Giza, Egypt.

DATA AVAILABILITY

The data supporting the findings of the article is available within the article.

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