Toxicology Reports 13 (2024) 101848

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Diosmin and Coenzyme q10: Synergistic histopathological and functional protection against doxorubicin-induced hepatorenal injury in rats

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ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Doxorubicin
Diosmin
Co-enzyme Q10
hepatorenal toxicity
rats

ABSTRACT

Doxorubicin (DOX) is a cytotoxic anthracycline used to treat a variety of cancers. Cardiotoxicity, hepatotoxicity, and nephrotoxicity are adverse effects of DOX, that limit prognosis. The study aims to determine if diosmin (DIOS) and coenzyme Q10 (CoQ10) alone or in combination protect rats against DOX-induced liver and kidney damage. Adult male rats were assigned randomly in five groups. An intraperitoneal injection of DOX (2.5 mg/kg) was given to the DOX group every other day for three weeks, whereas a normal control group received the vehicle. Diosmin group received oral DIOS (100 mg/kg), Co-Q10 group received oral CoQ10 (10 mg/kg) and combination group received oral DIOS and CoQ10 daily for three weeks concomitantly with DOX. Sera and tissues were obtained 24 hours after last DOX injection. Serum aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, total bilirubin and direct bilirubin were detected with hepatic and renal reduced glutathione (GSH), malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa-B (NF-κB). Histopathology and morphometry of liver and kidney were assessed. DOX exerted significant hepatorenal toxicity via elevation of liver and kidney functions, inducing oxidative stress by reducing GSH and elevating MDA, triggering renal and hepatic TNF-α and NF-kB. DIOS and CoQ10 modulated hepatic and renal functions, oxidative stress and inflammatory biomarkers. DIOS-CoQ10 combination treatment showed significant improvement in histopathology of liver and kidney along with morphometry compared to DOX group. In conclusion, combining DIOS and CoQ10 exhibited synergistic protective activity against DOX-induced hepatic and renal insult via their antioxidant and anti-inflammatory properties.

1. Introduction

Doxorubicin (DOX) or Adriamycin, is an anthracycline antibiotic which is recognized as an effective anticancer agent for the management of several types of cancers [1]. Chemotherapy, including DOX, predominantly promotes cell death by triggering necrosis [2]. However, the limitations and challenges associated with current cancer treatments have driven the pursuit of new therapeutic strategies [3]. Unfortunately, the clinical use of DOX has been deterred due to dose-related multi-organ toxicities such as the heart, the kidney, and the liver [4]. DOX induces a state of oxidative stress due to the metabolism of its quinone to a semi-quinone radical that leads to reactive oxygen species (ROS) by redox cycling triggering oxidative damage to cell membranes, DNA, and proteins [5]. Hepatotoxicity [6] and nephrotoxicity are serious side effects of DOX [7]. DOX has been observed to induce hepatocyte damage by cell cycle arrest [8], which in turn inhibits the self-regenerating capacity of the liver [9].

DOX-induced nephrotoxicity is manifests as nephropathy,

Abbreviation: DOX, Doxorubicin; DIOS, Diosmin; CoQ10, Coenzyme Q10; AST, Aspartate Transaminase; ALT, Alanine Transaminase; GSH, Glutathione; MDA, Malondialdehyde; TNF-α, Tumor Necrosis Factor-alpha; NF-κB, Nuclear Factor kappa-B; ELISA, Enzyme-Linked Immunosorbent Assay; QPCR, Quantitative Polymerase Chain Reaction; H&E, Hematoxylin and Eosin; CI, Combination Index; SEM, Standard Error of the Mean; ANOVA, Analysis of Variance; DBIL, Direct Bilirubin; TBIL, Total Bilirubin; I.p., Intraperitoneal; RNA, Ribonucleic Acid; RT-qPCR, Reverse Transcription Quantitative Polymerase Chain Reaction.

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https://doi.org/10.1016/j.toxrep.2024.101848
Received 16 October 2024; Received in revised form 23 November 2024; Accepted 29 November 2024
Available online 30 November 2024

proteinuria, and glomerulosclerosis [10]. As the ability of the kidney to regenerate and heal is limited, DOX causes almost irreversible kidney damage. The excessive production of ROS and RNS after DOX metabolism increases the susceptibility for lipid peroxidation, DNA damage, and decreases cellular thiols and vitamin E levels as reported earlier [11] that exacerbates DOX toxicity and inducing calcium overload mitochondrial dysfunction [12]. Significant reports link hepatorenal injury to oxidative stress [13–16]. DOX can correspondingly stimulate inflammatory pathways [17] by increasing the expression of nuclear factor kappa-B (NF-κB) [18]. The relationship between DOX and NF-kB, a transcriptional factor that regulates genes that encode apoptosis and inflammatory cytokines, interleukins, tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (i-NOS) [19,20]. These inflammatory pathways play an important role in DOX-induced hepatorenal disruption [21]. Herein, compounds with both antioxidant and anti-inflammatory effects required could protect against the hepatorenal damage associated with DOX. In this context, the exploration of new drugs from traditional medicinal remedies remains an attractive and continually advancing approach [22]. Medicinal plants are considered valuable for cancer prevention and treatment due to their anti-tumor compounds, ability to boost immune function, and potential to delay cancer onset [23]. They also offer a promising alternative or supplement to conventional cancer therapies by reducing or preventing side effects [24], (https://patents.justia.com/patent/10568873), https://patents.justia.com/patent/20200276133, https://patents.justia.com/patent/10933076)

Flavonoids are polyphenolic tricyclic secondary metabolites that are abundant in plants [25]. Diosmin (diosmetin 7-O-rutinoside) is a natural flavone glycoside. It is also called as venosmine (Teucrium gnaphalodes). It is a flavone glycoside derived from hesperidin that is abundantly present in citrus fruits [26]. It has bioactive effects, including an antioxidant anti-inflammatory and anti- apoptotic activity [19, 27, 28]. Flavonoids are known to decrease oxidative stress by lowering malondialdehyde (MDA) while enhancing the total antioxidant status levels in the body [29]. Moreover, diosmin displayed protective activity against liver and kidney injury, as well as hepatocellular carcinoma [28, 30, 31]. Diosmin, like other natural products have been utilized to develop potent anticancer drugs and supportive agents for chemotherapy [32].

The chemopreventive effects of antioxidant compounds have been proposed against DOX-induced toxicity [8]. The only naturally occurring and endogenously synthesized lipid-soluble antioxidant is (Co-Q10), also known as ubiquinone [33]. CoQ10 minimized cardiotoxicity related to Dox therapy [34], and Dox-induced nephrotoxicity [35]. CoQ10 reported protective effect against ochratoxin A toxicity in liver tissue [36]. CoQ10 is used as a dietary supplementation as well as an adjunct therapy with medication in a number of conditions, including cardiovascular diseases, cancer, muscular neurodegenerative disorders, and diabetes [37]. Hence, the current investigation was undertaken to assess the protective and synergistic activity effect of diosmin and CoQ10 against DOX-induced liver and kidney injury in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar Albino rats (n = 40), weighing 200–250 g, were purchased from the National Research Centre's animal house (Dokki, Giza, Egypt). Rats were kept at suitable laboratory conditions and at standard housing facilities (23 ± 2 ∘C, 45 ± 5 % humidity and a 12 /12-h light/dark cycle). They were fed standard pellet chow and permitted free access to water using special dropper-tipped bottles.

2.2. Chemicals

Doxorubicin, diosmin and Coenzyme Q10 were obtained from Sigma chemical company, St Louis, MO, USA. Rat TNF-α ELISA kit was obtained from **R&D Systems, USA** (Catalog Number: DY510–05). Total bilirubin (TBIL) and direct bilirubin (DBIL) in rat sera were obtained from BioVision, Inc., USA (**Total and Direct; Cat. No: K553)**. Other chemicals were obtained either from Sigma chemical company or commercial suppliers, unless otherwise mentioned.

2.3. Experimental design

Forty male Wistar rats, weighing 200–250 g ($n = 8$), were divided into five groups. **Group I** considered as the Normal control group; rats received intraperitoneal injection of normal saline and oral formulation of 1 % carboxymethylcellulose. In **Group II**, Doxorubicin (DOX, Sigma Co., St. Louis, MO, USA) group; rats were injected with DOX at a dose of 2.5 mg/kg i.p., every other day for 3 weeks, as described previously [38]; this dose regimen was used to develop DOX-cumulative toxicity as described previously [39]. **Group III, IV and V**, rats were treated orally with 100 mg/kg of diosmin (DIOS, Sigma Co., St. Louis, MO, USA) [40], 10 mg/kg Coenzyme Q10 (CoQ10, Sigma Co., St. Louis, MO, USA) prepared in 1 % carboxymethylcellulose [41], and Dios+CoQ10 on a daily basis concurrently with DOX for three weeks. At the end of the experimental schedule, **the body weight** of each animal was recorded. Throughout the research study, no experimental animal group experienced any mortality. Rats were euthanized with ketamine/xylazine solution (50 mg/kg- 5 mg/kg, i.p) [42]. Blood samples were collected from retro-orbital plexus and left to clot then centrifuged for 10 min at 5000 rpm. Sera were stored at −20 °C until biochemical analysis.

2.4. Liver and kidney tissues preparation

Liver lobes and both kidneys from each rat were detached, weighed and directly placed into liquid nitrogen (-80 °C) for Realtime quantitative PCR (QPCR) and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was kept at −20°C until biochemical analysis. The liver and kidney of all groups were dissected and inspected grossly for any changes and fixed immediately in 10 % neutral formalin for histological and morphometric studies.

2.5. Specimen collection, total RNA extraction and reverse transcription

The RNAlater RNA Stabilization Reagent (Qiagen) was used for preserving specimens used for gene expression analysis using RT-qPCR, following the manufacturer's recommendations. Resected testicles were cut longitudinally into less than 0.5 cm specimens and submerged immediately in approximately 10 volumes of RNAlater RNA Stabilization Reagent (Qiagen).

Specimens were incubated overnight at 4°C before storage at −80°C until RNA extraction process started. Thirty milligrams RNA-stabilized specimens were used for total RNA extraction using QIAzol Lysis Reagent (Qiagen) according to the manufacturer's directions. Forty microliters nuclease-free water were used to elute total RNA. All tRNA samples were stored at −80°C. Total RNA integrity was verified by agarose gel electrophoresis. Total RNA purity was verified by A260/A280 nm absorption ratio > 1.85. For reverse transcription, 1 μg total RNA was used. The QuantiTect Reverse Transcription Kit (Qiagen) was used according to the manufacturer's directions. The final volume of reverse transcription reactions was 20 μl. The reverse transcription method involved genomic RNA removal step using the gDNA Wipeout Buffer included in the kit.

2.6. Real-time quantitative PCR (QPCR) of NF-κB-p65 mRNA

RT-qPCR was used to measure the mRNA levels of Nfe2l2 based on SYBR Green I chemistry. The reference gene Actb was used to normalize the mRNA levels of the Nfe2l2. The predesigned QuantiTect Primer

Assays from Qiagen were used for both Nfe2l2 (catalogue number QT00183617; and assay name Rn_RGD:620360_1_SG) and Actb (catalogue number QT00193473; and assay name Rn_Actb_1_SG). QPCR was performed in duplicate reactions using QuantiTect SYBR® Green PCR Kit (Qiagen) according to manufacturer's directions in a final reaction volume of 25 μl. Reactions were pipetted in semi-skirted 96-well plates (Eppendorf AG) and sealed with optical adhesive PCR film (Eppendorf AG). Reactions were run using StepOnePlus ™ Real-Time PCR System (Applied Biosystems ™). The denaturation step was performed at 95°C for 15 min, followed by 40 PCR cycles with the following specifications: 94°C for 15 sec, 55°C for 30 sec, 72°C for 30 sec. No template controls (NTCs) were included in all the assays. At the end of each QPCR experiment, a meltimg curve was generated using the following thermal profile: 95°C for 1 min, 55°C for 30 sec, 95°C for 30 sec to test for the specificity of each assay. Applied Biosystems® Real-Time PCR software version 3.2 was used for QPCR data analysis. The relative mRNA expression levels of Nfe2l2 was obtained using the comparative delta dela CT method [1] after normalization to the expression of the reference gene; Actb yielding a ΔCt value. The −ΔΔCt value was then calculated by subtracting the average ΔCt value of Nfe2l2 samples from control group from the respective ΔCt values of treated animals. The −ΔΔCt values were then used to calculate the relative mRNA expression ratios ($2^{-\Delta\Delta Ct}$).

2.7. Biochemical investigations

Serum levels of liver function enzymes; alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assayed. Serum kidney biomarkers; creatinine and urea were analyzed colorimetrically (**Bio-diagnostics, Giza, Egypt**), according to their standard procedures. Levels of total bilirubin (TBIL) and direct bilirubin (DBIL) in rat sera were assessed using colorimetric assay Kit for Bilirubin (**Total and Direct; Cat. No: K553, BioVision, Inc., USA**). Hepatic and renal homogenate contents of malondialdehyde (MDA) and reduced glutathione (GSH) were detected using colorimetric assay kits according to the manufacturer's instructions (**Biodiagnostic, Egypt**). The levels of hepatic and renal tumor necrosis factor-α (TNF-α) were determined using rat TNF-α ELISA kit (**R&D Systems, USA**) according to the manufacturer's recommendations.

2.8. Histopathological examination of hepatic and renal tissues

Both liver and kidney tissues were immediately dissected, inspected visually for any abnormalities, and fixed in 10 % formalin saline. Hematoxylin and eosin (H&E) was used to stain sections of the liver and kidney that were cut to a thickness of 4 m before being inspected under a light microscope for histological alterations and imaging.

2.9. Morphometric analysis of hepatic and renal tissues

The Leica Qwin 500 Image Analyzer (LEAICA Imaging Systems Ltd, Cambridge, England) was used to process hepatic and renal sections stained with hematoxylin and eosin for morphometric measures. The Leica DM-LB microscope and JVC colour video camera used in this image analyzer were connected to a computer system. The slides were examined under low magnification power x50 to look for areas of pathological abnormalities. Measurements were performed using x200 and x400 magnification. A computerized image analysis system was used for the morphometric study.

2.10. Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey's multiple comparison post hoc test, and are shown as means standard error of the mean (SEM) of eight experiments. When $p<0.05$, a difference was considered significant. The software package GraphPad Prism, version 9 (GraphPad Software, USA), was used for data analysis.

The combination-index (CI), a numerical measure of the pharmacological interaction between two medications as previously defined by ***Chou and Talalay*** [43], was used to assess the interaction between DIOS and CoQ10 in this study. Using CompuSyn 1.01 software (ComboSyn, Inc., Paramus, NJ, USA), the CI values of interactions between DIOS and CoQ10 were evaluated [43]: CI< 1 was considered to represent a synergistic impact [44]. This methodology has been applied in a number of earlier research [45–47].

3. Results

3.1. Effect of DIOS and CoQ10 on relative liver and kidneys weights

DOX injection at 2.5 mg/kg every other day for three weeks exerted significant changed relative liver and kidneys weights significantly. Oral treatment of DOX-injected rats with DOX, CoQ10 or both significantly improved liver and kidney weights compared to DOX group (Table 1).

3.2. Effect of DIOS and CoQ10 on serum liver function biomarkers

Dox-induced hepatorenal toxicity was evident by significant increase in serum AST, ALT, direct and total bilirubin as well as the elevation in serum creatinine and urea by 1.6, 3.5, 2.6, 2.4, 2.2 and 2.4-fold-increase compared to normal control (Table 2). Treatment with DIOS or CoQ10 significantly mitigated the elevation in liver and kidney indices compared to DOX group. Additionally, combination of both DIOS and CoQ10 exerted potent ameliorative effect on liver and kidney indices of DOX-treated rats recording 13, 24, 55, 49, 34 and 28 % decline in serum AST, ALT, direct and total bilirubin, serum creatinine and urea, respectively, compared to DOX. Of note, the effect of DIOS and CoQ10 combined was significantly different from either treatment alone. Remarkably, the CI indicated that DIOS in combination with CoQ10 showed synergistic interactions on ALT, urea and creatinine where CI values were less than 1 (Table 2).

3.3. Effect of DIOS and CoQ10 on liver oxidative stress biomarkers

Intraperitoneal injections of DOX at 2.5 mg/kg every other day for three weeks significantly affected the antioxidant status in both liver and kidney. Results indicated significant decrease in hepatic and renal GSH by 60 and 71 %, respectively with a 3- and 3.5-fold increase in MDA of hepatic and renal tissues, respectively, of DOX-treated animals by compared to normal counterparts (Fig. 1). Daily oral administration of DIOS, or CoQ10 significantly modulated the oxidative stress induced by DOX in rat liver and kidney compared to DOX-injected rats. Combination treatment markedly improved hepatic and renal GSH by 90 and 133 % increase, respectively, while reduced hepatic and renal MDA by 50 and 60 % decline, respectively, compared to DOX group. Remarkably, the CI indicated that DIOS in combination with CoQ10 showed synergistic interactions hepatic and renal GSH where CI values were less than 1 (Fig. 1).

3.4. Effect of DIOS and CoQ10 on liver inflammatory biomarkers

Following DOX injection, the inflammatory cytokine; tumor necrosis factor-alpha (TNF-α) and its inducible transcription factor; nuclear factor (NF)-κB were significantly elevated in rat liver and kidney by 3.7- and 2.5-fold increase for hepatic and renal TNF-α, respectively, compared to normal. Further, the expression of hepatic and renal NF-κB recorded 1.7- and 3-fold increase, respectively, in DOX-treated rats compared to normal animals (Fig. 2). Interestingly, rats treated with DIOS or CoQ10 or their combination displayed significant reduction of hepatic and renal levels of TNF-α compared to DOX. Similarly, the expression of NF-κB in both liver and kidney of DOX-treated rats was

Table 1
Effect of DIOS and CoQ10 on relative liver and kidneys weights in DOX-treated rats.

Groups Parameters	Normal control	DOX	DIOS+ DOX	Co-Q10 + DOX	DIOS+Co-Q10 + DOX
Relative Liver Wt.	0.031 ± 0.0005	0.049^{a} ± 0.0015	0.039^{ab} ± 0.0012	0.042^{ab} ± 0.0029	0.033^{abc} ± 0.0023
Relative Kidney Wt.	0.051 ± 0.0005	0.084^{a} ± 0.001	0.062^{ab} ± 0.001	0.079^{ab} ± 0.002	0.059^{abc} ± 0.001

Hepatorenal toxicity was induced in rats by intraperitoneal injection of doxorubicin (DOX; 2.5 mg/kg, i.p) every other day for three weeks concurrently with daily oral administration of diosmin (DIOS, 100 mg/kg), Coenzyme Q10 (CoQ1010 mg/kg) or their combination. Twenty-four hours after last DOX injection, animals were weighed then the liver and kidney tissues were collected and weighed after sacrifice. Relative liver and kidney weights were then calculated. Data expressed as the mean ± SEM. Statistical analyses were carried out using ANOVA followed by Tukey's multiple comparisons. test. [a], $P < 0.05$ vs. the normal control, [b], $P < 0.05$ vs. DOX treated rats, [c], $P < 0.05$ vs. DIOS or CoQ10 group.

Table 2
Effect of DIOS and CoQ10 on serum hepatic and renal functions biomarkers in DOX-treated rats.

Groups Parameters	Normal control	DOX	DIOS+ DOX	Co-Q10 + DOX	DIOS + CoQ10 + DOX
Serum AST (U/dl)	98.8 ± 2.4	162.3^{a} ± 1.1	147.5^{ab} ± 0.76	150.5^{ab} ± 0.76	139.7^{abc} ± 1.02
Serum ALT (U/dl)	38.7 ± 2.2	136.5^{a} ± 0.92	116.3^{ab} ± 1.15	127.8^{ab} ± 1.01	$103.4^{abc\#}$ ± 1.2
Serum DBil (mg/dl)	0.11 ± 0.005	0.29^{a} ± e0.014	0.16^{ab} ± 0.004	0.20^{ab} ± 0.11	0.13^{abc} ± 0.006
Serum TBil (mg/dl)	0.24 ± 0.007	0.59^{a} ± 0.01	0.34^{ab} ± 0.006	0.40^{ab} ± 0.012	0.30^{abc} ± 0.01
Serum Creatinine (mg/dl)	0.23 ± 0.008	0.50^{a} ± 0.014	0.40^{ab} ± 0.007	0.47^{ab} ± 0.014	$0.33^{abc\#}$ ± 0.016
Serum Urea (g/dl)	20.98 ± 0.46	51.50^{a} ± 0.99	42.33^{ab} ± 0.88	46.50^{ab} ± 1.33	$37.83^{abc\#}$ ± 1.35

Hepatorenal toxicity was induced in rats by intraperitoneal injection of doxorubicin (DOX; 2.5 mg/kg, i.p) every other day for three weeks concurrently with daily oral administration of diosmin (DIOS, 100 mg/kg), Coenzyme Q10 (CoQ1010 mg/kg) or their combination. Twenty-four hours after last DOX injection, blood samples were collected and serum was used to evaluate aspartate transaminase (AST), alanine transaminase (ALT), direct bilirubin (DBIL), total bilirubin (TBIL), serum creatinine and serum urea. Data expressed as the mean ± SEM. Statistical analyses were carried out using ANOVA followed by Tukey's multiple comparisons. test. [a], $P < 0.05$ vs. the normal control, [b], $P < 0.05$ vs. DOX treated rats, [c], $P < 0.05$ vs. DIOS or CoQ10 group. (#) Indicates synergistic interaction using coefficient drug index (CDI).

significantly reduced by DIOS or CoQ10 that further recorded normal levels. While combined, DIOS and CoQ10 recorded significantly low levels of hepatic and renal NF-κB expression compared to either alone, displaying 75 and 81 % reduction compared to DOX group. Notably, the CI indicated that DIOS in combination with CoQ10 showed synergistic interactions expression of hepatic and renal NF-κB as well as renal TNF-α where CI values were less than 1 (Fig. 2).

3.5. Effect of DIOS and CoQ10 against DOX-Induced hepatorenal histopathological alterations in rats

The histological analysis of the control liver reveals normal hepatic architecture; the blood sinusoids that separate the cords have normal central veins. Liver tissues of DOX-treated group display massive dilatation of central vein and congestion as well as presence of inflammatory infiltrate with some vascular degeneration. Treatment with either DIOS or CoQ10 show marked reduction of DOX insult on hepatic tissue. Groups treated with combination of DIOS and CoQ10 show normal central vein with normal hepatocyte (Fig. 3).

The examination of control kidney group with light microscope revealed normal histological structure of glomeruli and tubules. DOX-treated groups show dilated edematous tubules with multiple vacuoles, some distorted and damaged tubules with normal glomeruli. DIOS-treated group shows marked improvement in tubular damage, similarly groups that treated with the combination of DOX and CoQ10. These groups treated with combination of DIOS and CoQ10 show reversal of damage and restoration of normal renal morphology (Fig. 4).

3.6. Effect of DIOS and CoQ10 against DOX-Induced hepatorenal morphometric alterations in rats

Hepatic morphometric measurements were performed to examine the central vein area by using measure feature program. The percentage of hepatic central vein area over the whole observed slide was assessed and expressed as mean. Morphometric measurement of rat hepatic tissue showed significant increase in the central vein area compared with that of control group. Area of the central vein in DIOS- or CoQ10-treated groups exhibited significant decrement compared to DOX group. Whereas the area of central vein significantly decreased in combination therapy of DIOS and CoQ10 compared to DOX, DIOS or CoQ10-treated groups (Fig. 3 and Table 3).

Renal morphometric measurements were done to measure the area of proximal tubules using interactive measurements program. The proximal tubular area over the whole observed slide was assessed and expressed as mean. Morphometric measurement of DOX treated group displayed increased area of proximal tubules compared to control group. Treatment with either DIOS or CoQ10 exerted significant improvement of proximal tubular areas compared to DOX group. While the mean of proximal tubular area in combination therapy of DIOS and CoQ10 revealed significant reduction compared to DOX, or either treatment (Fig. 4 and Table 3).

4. Discussion

Doxorubicin is considered a potent and effective anticancer in solid tumors [48]. Yet, it is the most toxic among anthracyclines; the reason behind its limited clinical use [48,49]. DOX toxicity is substantially associated with induction of oxidative damage [50] either in acute toxicity following single dose injection or chronic toxicity following multiple low doses of DOX over 2–12 week-experiment affecting liver, kidney and heart in animals [51,52].

By injecting 2.5 mg/kg of DOX every other day for three weeks, this study examined the long-term model of DOX-induced chronic toxicity on the livers and kidneys of rats. The long-term model of DOX, established in this study, was to consider what is called cumulative dose toxicity reported clinically in patients treated continuously or rechallenged with DOX [53]. As generally accepted in clinical settings that the degree of DOX-induced cardiotoxicity is greatly related to the increase in cumulative dose [54]. Unfortunately, the administration of DOX beyond the recommended cumulative dose is a therapeutic option for the treatment of chemotherapy-sensitive advanced sarcomas due to lack of other effective modalities [55,56]. Whereas in some of those patients, DOX is discontinued due to advanced cardiotoxicity and eventually died of uncontrolled tumor growth with no drug-related deaths reported [55].

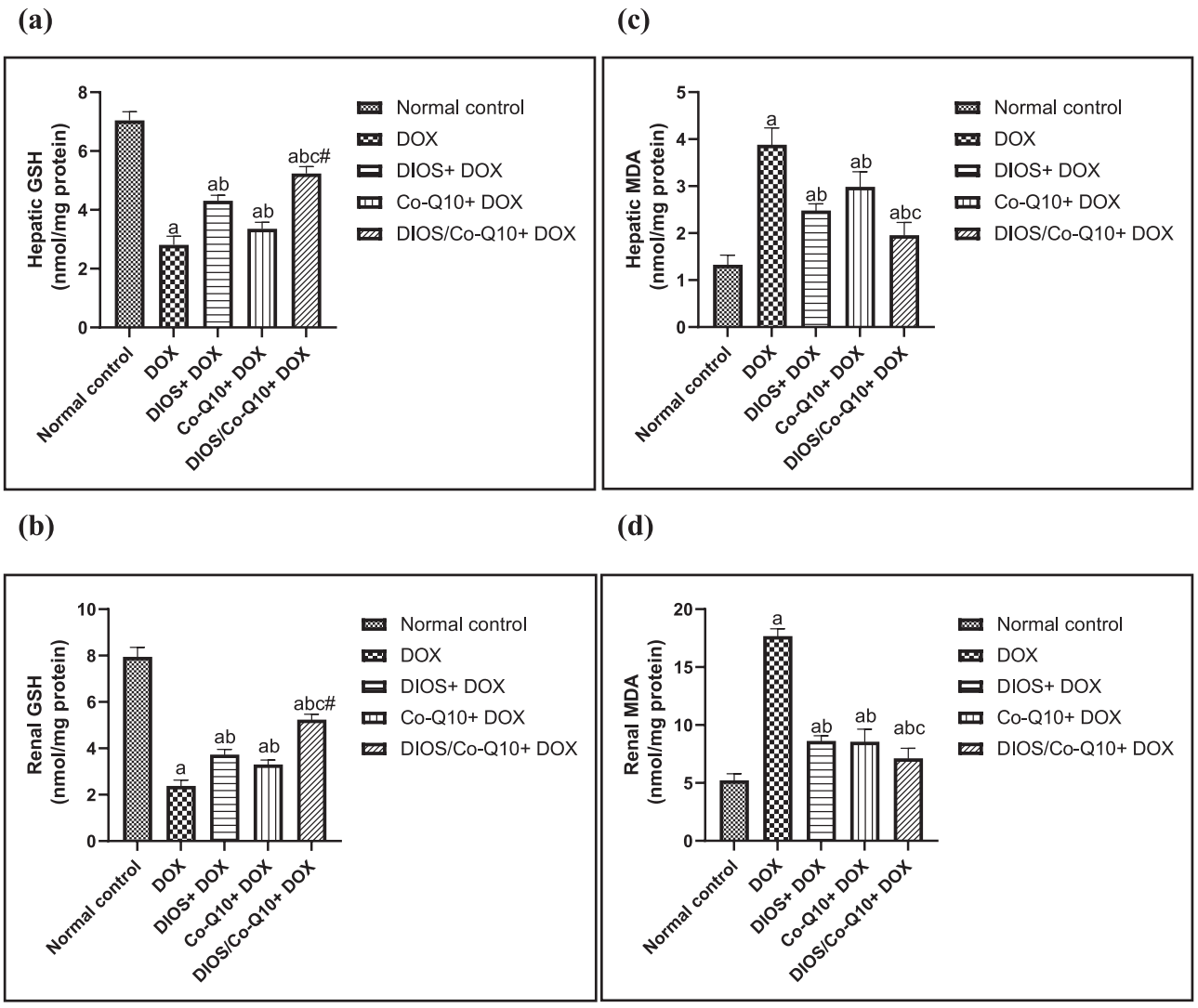

Fig. 1. Effects of DIOS and CoQ10 against DOX-Induced hepatorenal Oxidative Stress, hepatic GSH (a), hepatic MDA (b), renal GSH (c), and renal MDA (d) in male Wistar rats. Data expressed as the mean ± SEM. Statistical analyses were carried out using ANOVA followed by Tukey's multiple comparisons. test. [a], $P < 0.05$ vs. the normal control, [b], $P < 0.05$ vs. DOX treated rats, [c], $P < 0.05$ vs. DIOS or CoQ10 group. (#) Indicates synergistic interaction using coefficient drug index (CDI).

Herein, the present study indicated DOX cumulative-dose hepatorenal-induced toxicity in rats after three weeks. As both liver and kidney relative weights and toxicity indices markedly elevated in DOX-treated animals such as serum aminases (ALT and AST) that have been contemplated as index of hepatic injury [57] and clinically approved markers of liver injury [58]. Similarly, current results demonstrated high serum levels of creatinine and urea accounting for DOX-induced nephrotoxicity [59,60]. Liver cell injury prompts the release of these enzymes into the bloodstream. Elevated plasma levels of these liver enzymes indicate a toxic impact on the liver [61].

Liver-kidney interaction is well-established as liver-associated kidney disease, and the spectrum include both acute and chronic kidney diseases [62]. The present study indicated a state of hyperbilirubinemia in rats evidenced by elevation in both serum direct and total bilirubin following DOX injection that further confirm intrahepatic toxicity that can lead to either unconjugated or conjugated hyperbilirubinemia [63]. This state of hyperbilirubinemia may add on further kidney damage by DOX nephrotoxic effect or intratubular deposition of bilirubin or both.

On the other hand, protection against DOX-hepatorenal alteration in organs' weight and toxicity indices by treatment with DIOS at 100 mg/kg, or CoQ10 at 10 mg/kg or in combination was reported in the present study. Several natural compounds reported hepatic and renal protection against DOX in vivo [39, 59, 64, 65]. This indicates that DIOS [40,66] and CoQ10 [41,67] provide protection against liver and kidney toxicity as stated earlier.

In the current study, a significant decline in hepatic and renal GSH and a significant incline in hepatic and renal MDA were demonstrated following DOX injection in accordance with previous studies [68]. Confirming DOX-induced oxidative damage to liver and kidney tissues in animals [21,69], suggestive of free radical formation [70], lipid peroxidation [71] and a considerable decrease in hepatic and renal GSH levels in response to DOX [40,66]. Supplementation with DIOS or CoQ10 or combined attenuated DOX alterations in hepatic and renal GSH and MDA. As increment in the endogenous antioxidant, GSH effectively guarantees normal cellular function via detoxification of ROS [72], thus Diosmin (DIOS) and Coenzyme Q10 (CoQ10), herein reported significant hepatorenal protection owing to their antioxidant potentials. Following DOX injection, changes in GSH levels and CAT activity in the hepatic system were mitigated by diosmin pretreatment [40]. Further, DIOS restored MDA levels to baseline and mitigated the changes in GSH content, SOD expression, and CAT activity following DOX administration, which emphasizes the antioxidant properties of this compound [66, 73]. CoQ10 acts as a soluble antioxidant and free radical [74] thus stabilizes the cell membrane and the intracellular membranes by

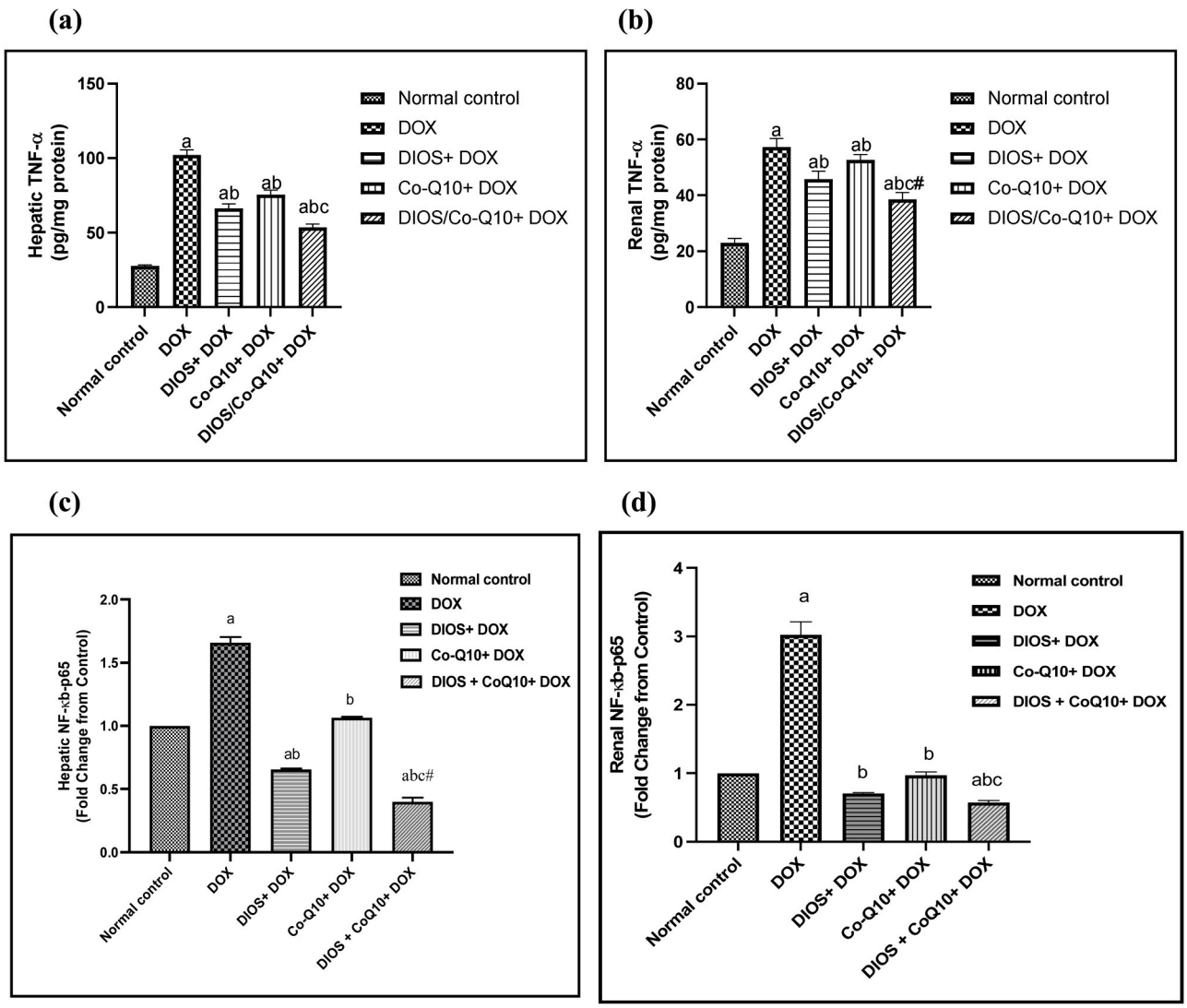

Fig. 2. Effects of DIOS and CoQ10 against DOX-Induced hepatorenal changes in Inflammatory Markers, hepatic TNF-α (a), renal TNF-α (b), hepatic NF-KB (c), and renal NF-KB (d), in male Wistar rats. Data expressed as the mean ± SEM. Statistical analyses were carried out using ANOVA followed by Tukey's multiple comparisons. test. [a], $P < 0.05$ vs. the normal control, [b], $P < 0.05$ vs. DOX treated rats, [c], $P < 0.05$ vs. DIOS or CoQ10 group. (#) Indicates synergistic interaction using coefficient drug index (CDI).

protecting the phospholipids of the membranes from peroxidation [75]. In addition, CoQ10 revives key antioxidants [76]. Previously, CoQ10 administration attenuated DOX-induced increase in MDA and NO production along with increasing GSH content together with the activity of GPx, SOD, and CAT in rats [67, 77, 78]. It is noteworthy, that combined treatments exerted remarkable synergistic antioxidant activity compared to either DIOS or CoQ10.

Furthermore, DOX induces nephrotoxicity as it accumulates in the kidney leading to pronounced permeability of glomerular capillary and tubular degeneration [79]. Further, DOX harms other tissues such as the heart and the liver that may alter both structure and function of main organs, henceforth circumlocutory causing nephropathy. Nevertheless, antioxidants proved to have chemo-preventive effects against DOX-multi-organ insult [80].

Many factors can trigger inflammatory response including harmful chemicals, pathogens, and injured cells [81]. During inflammation, activated macrophages undergo phagocytosis, and produce cytokines and growth factor [82]. Significant number of studies related DOX and nuclear factor kappa B (NF-κB), a transcriptional factor that regulates genes that encode apoptosis and inflammatory cytokines [83]. **El-Moselhy and El-Sheikh** posit that DOX-induced oxidative stress also induces TNF- release, which would activate additional signaling pathways, such as the inflammatory (NF-κB) pathways [13].

The involvement of the inflammatory pathway in DOX-mediated hepatotoxicity [84] and nephrotoxicity [85] is well documented Current data supports the activation of NF-κB and proinflammatory cytokines such as TNF-α in rat hepatic and renal tissues post DOX administration. NF-κB is a nuclear transcription factor that plays a pivotal role in the pathophysiology of drug-induced hepatotoxicity [39]. Moreover, NF-kB is regarded as a key factor in various pathological processes, recognized for its role in connecting chronic inflammation and oxidative stress to the development of disease-related inflammation [86]. There is cross-talk between proinflammatory cytokine overproduction and oxidative stress [87]. Alongside its pro-inflammatory functions, TNF-α acts as an anti-tumor cytokine and has been reported to stimulate NF-κB activation [88]. DOX creates a microenvironment that triggers the activation of NF-κB and pro-inflammatory cytokines, such as TNF-α and IL-6, with inhibition of anti-inflammatory cytokines such as IL-10 [89] due to increased oxidative stress and depleted intracellular antioxidants [90]. Diosmin and CoQ10 significantly curbed the activation of hepatic and renal NF-κB expression and hindered the elevation in hepatic and renal levels of TNF-α. Combination treatment

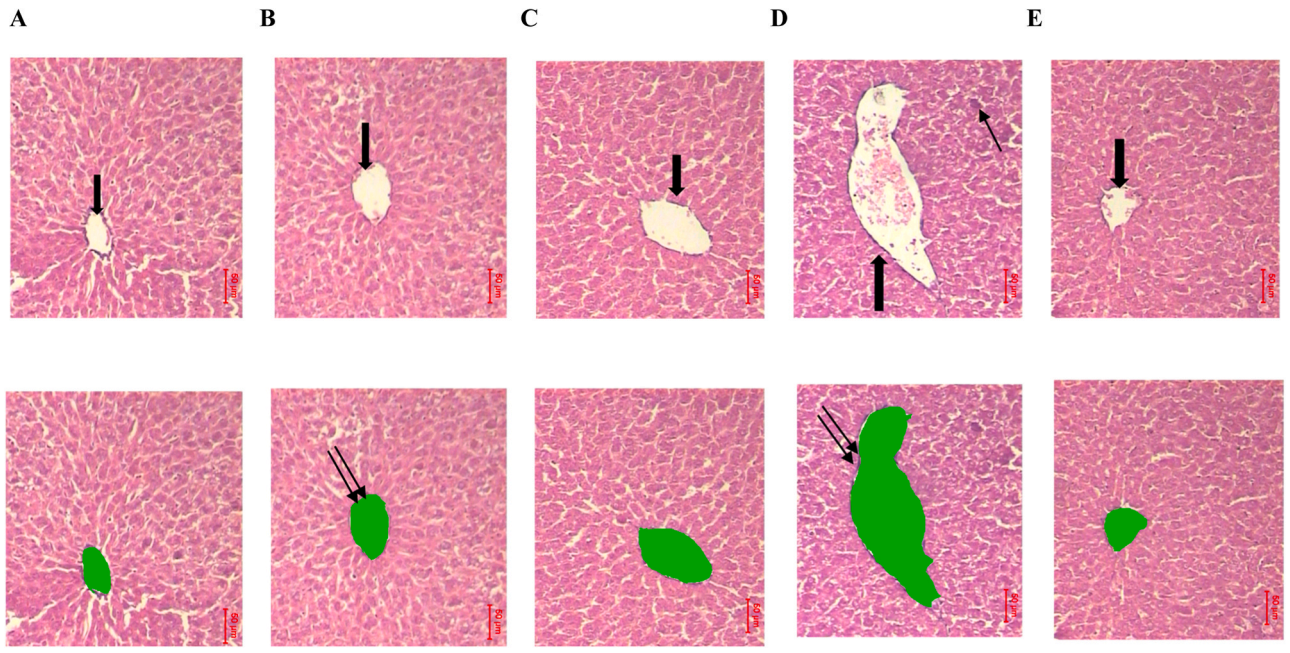

Fig. (3). Effects of Diosmin and CoQ10 against DOX-Induced hepatic histopathological and morphological alterations in male Wistar rats. A photo micrograph of rat hepatic tissue showed the following: (A) Control rat liver tissue exhibited normal architecture with intact hepatic cords and central vein. (B) Doxorubicin-treated rat liver tissue displayed inflammation (thin arrow), congestion, and significant dilatation of the central vein (thick arrow). (C) Liver tissue from rats treated with doxorubicin and diosmin showed some central vein dilatation (thick arrow) but no congestion. (D) Similar findings were observed in rats treated with doxorubicin and CoQ10. (E) Liver tissue from rats receiving the combination therapy (doxorubicin, diosmin, and CoQ10) showed a normal central vein (thick arrow) and healthy hepatocytes, with the central vein area measured by morphometry (double arrow) at 200x magnification.

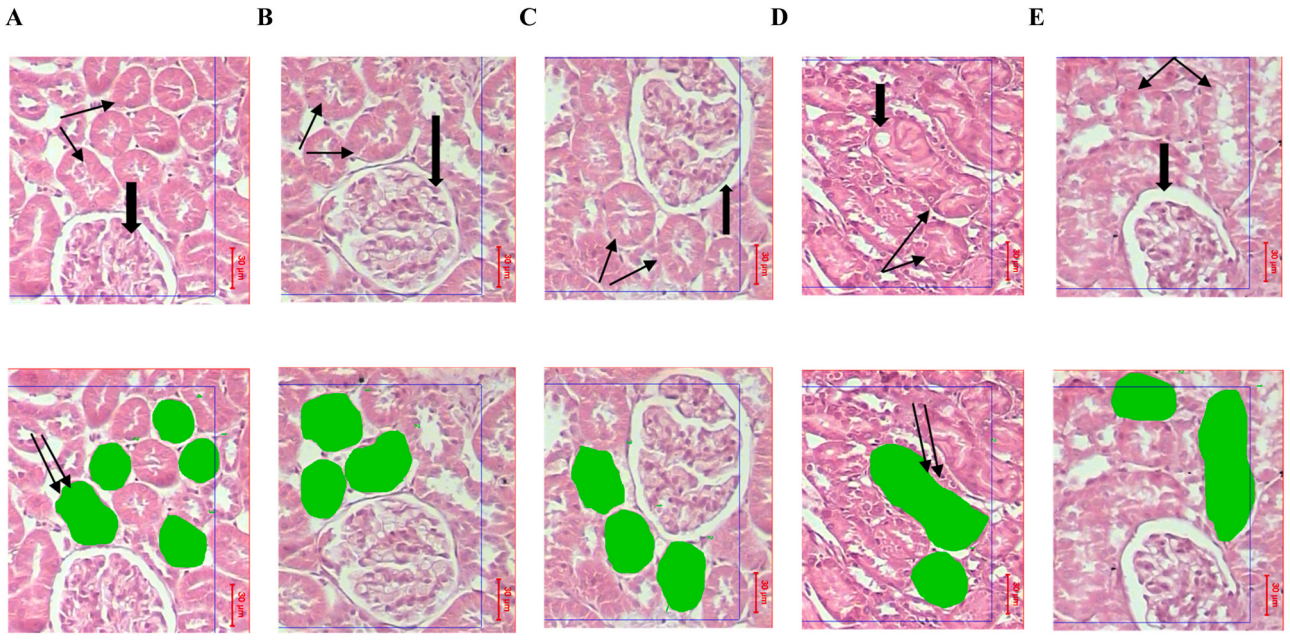

Fig. (4). Effects of Diosmin and CoQ10 against DOX-Induced renal histopathological and morphological alterations in male Wistar rats. A photo micrograph of rat renal tissue showed the following: (A) Control rat kidney tissue exhibited normal structure, with intact glomeruli (thick arrow) and tubules (thin arrow). (B) Renal tissue from rats treated with doxorubicin showed edematous tubules (thin arrow) and multiple vacuoles (thick arrow). (C) Renal tissue from rats treated with doxorubicin and diosmin showed improvement in tubular structure, with no vacuolation, and preserved glomeruli (thick arrow) and tubules (thin arrow). (D) Similar findings were observed in rats treated with doxorubicin and CoQ10, with intact glomeruli (thick arrow) and tubules (thin arrow). (E) Renal tissue from rats receiving the combination therapy (doxorubicin, diosmin, and CoQ10) showed normal renal morphology, with well-preserved glomeruli (thick arrow) and tubules (thin arrow). Green color highlighted the tubular area, which was measured by morphometry (double arrow) at 400x magnification.

with DIOS and CoQ10 significantly synergized the anti-inflammatory effect of either DIOS or CoQ10. Consistent with previous reports supporting the capability of a natural compound to hinder NF-κB activation [78,91]; diosmin has been reported to mitigate TNF-α, IL-1β, IL-6, and iNOS gene expressions in a dose-dependent manner [40,66]. Moreover, CoQ10 has been shown to have anti-apoptotic and inflammatory

Table 3
Effect of DIOS and CoQ10 on hepatic and renal tissues morphometric measurement.

Groups Parameters	Normal control	DOX	DIOS+ DOX	Co-Q10 + DOX	DIOS + CoQ10 + DOX
CV/ µm2	4051 ± 4.3	24480 ± 23.1*	15314 ± 94.8*@	14600 ± 82.1*@	4350 ± 18.9@#
PTa/ µm2	1272 ± 7.4	1995 ± 17.9*	1600 ± 19.6*@	1655 ± 22.9*@	1277 ± 9.7@#

Hepatorenal toxicity was induced in rats by intraperitoneal injection of doxorubicin (DOX; 2.5 mg/kg, i.p) every other day for three weeks concurrently with daily oral administration of diosmin (DIOS, 100 mg/kg), Coenzyme Q10 (CoQ1010 mg/kg) or their combination. Twenty-four hours after last DOX injection, tissues were isolated, fixed immediately in 10 % formalin saline. Section 4µm thick were cut from paraffin blocks. The sections were stained by hematoxylin and eosin (H&E) then examined with a light microscope for histological changes and imaging. The morphometric measurements were applied. Data expressed as the mean ± SEM. Statistical analyses were carried out using ANOVA followed by Tukey's multiple comparisons. test. *, $P < 0.05$ vs. the normal control, @, $P < 0.05$ vs. DOX treated rats. (#) Indicates synergistic interaction using coefficient drug index (CDI). CV=central vein area, PTa = proximal tubular area.

activities [92,93]. Further, CoQ10 significantly decreased cisplatin-induced overexpression of iNOS, NF-κB, caspase-3 and p53 in renal tissue [41]. To further confirm alterations in liver and kidney biochemical parameters and abnormal expression of inflammatory and apoptotic proteins, we assessed the histological alterations in the in response to DOX. DOX treatment distorted the architecture of both liver and kidney that is consistent with an earlier report [21]. Nonetheless, DIOS and CoQ10 restored the architecture of the liver and kidney compared with the DOX group.

Notably, flavonoids, including diosmin, possess inhibitory effects on breast cancer resistance protein in vitro and in vivo which further explain the increase in DOX's cytotoxicity when combined with DIOS [94]. In addition, CoQ10 did not affect the antineoplastic properties of DOX in breast cancer cell cultures [95] while also reported enhanced antitumor efficacy when co-administered with DOX [96].

The study is limited by the lack of analysis of phosphorylated NF-κB and its nuclear translocation, which are essential for understanding its activation in the inflammatory response. While total NF-κB and TNF-α measurements provide valuable insights, future research should include these parameters to better clarify the molecular mechanisms. Additional studies are also needed to explore the detailed antioxidant and anti-inflammatory pathways involved in the protective effects of DIOS and CoQ10 against DOX toxicity.

5. Conclusion

The combination of DIOS and CoQ10 holds significant potential not only to enhance the therapeutic efficacy of chemotherapy but also to reduce the multi-organ adverse effects associated with DOX in cancer patients. Our findings suggest that DIOS and CoQ10 may exert their protective effects through mechanisms involving oxidative stress and inflammation, which are central to DOX-induced toxicity. NF-κB, a key transcription factor involved in pro-inflammatory and apoptotic pathways, is likely activated by DOX, contributing to hepatorenal damage. By downregulating NF-κB activation, DIOS and CoQ10 can reduce inflammatory responses, thereby mitigating tissue injury and preserving cellular integrity in hepatic and renal tissues. Furthermore, the observed reduction in TNF-α levels indicates a suppression of upstream inflammatory signaling, further supporting the role of DIOS and CoQ10 in modulating inflammation-driven oxidative stress and apoptosis in DOX-induced toxicity as illustrated in Fig. 5.

In conclusion, the data from this study highlight the potential of

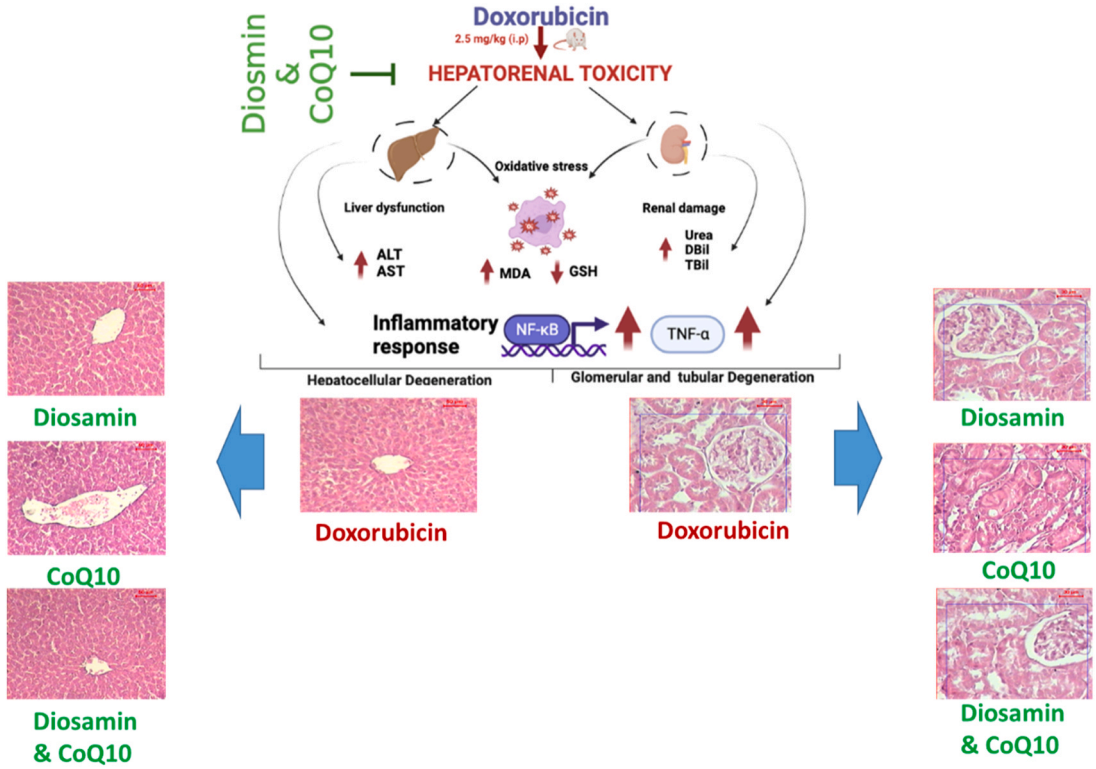

Fig. (5). A graphic summary shows the synergistic protective activity of Diosmin and Co-enzyme Q10 against doxorubicin-induced hepatorenal insult via modulation of oxidative stress, inflammatory responses and cytoprotection of liver and kidney functioning and architecture.

DIOS and CoQ10 as a synergistic intervention against DOX-induced hepatorenal injury, with beneficial effects on mitigating oxidative stress, restoring antioxidant balance, and alleviating inflammation.

Ethical approval and consent to participate

The experiment was conducted in accordance with the Medical Research Ethics Committee's (MREC) ethical standards for routine experimental animal studies, NRC, Egypt (In compliance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and the Guide for Care and Use of Laboratory Animals (U.S.—N.I.H. Publication No. 85–23, revised 1996).

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

D.F. Mansour and D.O. Saleh: have designed the study, collected the data of the work, drafted the article, revised the article and have finally approved the version to be published. M. Rady, I.M. Hashad, A.N. Abd-El Razik: have analyzed and interpreted the data, revised the article and have finally approved the version to be published.

Informed consent

The experiment is not a clinical study, so not applicable.

Consent for publication

I; the corresponding author, hereby, on behalf of all other authors, affirm that the content of this manuscript (in part or in full) has not been submitted or considered for publication elsewhere and is not currently being.

CRediT authorship contribution statement

Mona Rady: Writing – review & editing, Methodology, Investigation. **Amira Abd-El Razik:** Writing – original draft, Investigation. **Dalia Saleh:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Dina Mansour:** Writing – original draft, Methodology, Data curation, Conceptualization. **Ingy Hashad:** Writing – review & editing, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Facilities provided by Medical Research and Clinical Studies Institute - National Research Centre, Giza, Egypt and Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo, Egypt are acknowledged.

Data availability

Data will be made available on request.

References

[1] C. Carvalho, et al., Doxorubicin: the good, the bad and the ugly effect, Curr. Med Chem. 16 (25) (2009) 3267–3285.
[2] S. Abdu, N. Juaid, A. Amin, Effects of Sorafenib and Quercetin Alone or in Combination in Treating Hepatocellular Carcinoma: In Vitro and In Vivo Approaches 27 (22) (2022).
[3] S. Bouabdallah, A. Al-Maktoum, A. Amin, Steroidal Saponins: Naturally Occurring Compounds as Inhibitors of the Hallmarks of Cancer 15 (15) (2023).
[4] Z. Su, et al., Protective effects of madecassoside against Doxorubicin induced nephrotoxicity in vivo and in vitro, Sci. Rep. 5 (2015) 18314.
[5] J. Sun, et al., Myricitrin Protects against Doxorubicin-Induced Cardiotoxicity by Counteracting Oxidative Stress and Inhibiting Mitochondrial Apoptosis via ERK/P53 Pathway, Evid. Based Complement Altern. Med 2016 (2016) 6093783.
[6] C. Dai, et al., Lapatinib promotes the incidence of hepatotoxicity by increasing chemotherapeutic agent accumulation in hepatocytes, Oncotarget 6 (19) (2015) 17738–17752.
[7] A. Kumral, et al., Beneficial effects of carnosine and carnosine plus vitamin E treatments on doxorubicin-induced oxidative stress and cardiac, hepatic, and renal toxicity in rats, Hum. Exp. Toxicol. 35 (6) (2016) 635–643.
[8] S. Granados-Principal, et al., New advances in molecular mechanisms and the prevention of adriamycin toxicity by antioxidant nutrients, Food Chem. Toxicol. 48 (6) (2010) 1425–1438.
[9] N. Kassner, et al., Carbonyl reductase 1 is a predominant doxorubicin reductase in the human liver, Drug Metab. Dispos. 36 (10) (2008) 2113–2120.
[10] J. Armstrong, C.R. Dass, Doxorubicin Action on Mitochondria: Relevance to Osteosarcoma Therapy? Curr. Drug Targets 19 (5) (2018) 432–438.
[11] Y. Kalender, M. Yel, S. Kalender, Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin, Toxicology 209 (1) (2005) 39–45.
[12] M.C. Kockar, et al., N-acetylcysteine modulates doxorubicin-induced oxidative stress and antioxidant vitamin concentrations in liver of rats, Cell Biochem Funct. 28 (8) (2010) 673–677.
[13] M.A. El-Moselhy, A.A. El-Sheikh, Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity, Biomed. Pharm. 68 (1) (2014) 101–110.
[14] C. Ortiz, et al., Inhibition of the EGF receptor blocks autocrine growth and increases the cytotoxic effects of doxorubicin in rat hepatoma cells: role of reactive oxygen species production and glutathione depletion, Biochem Pharm. 75 (10) (2008) 1935–1945.
[15] N. Ashley, J. Poulton, Mitochondrial DNA is a direct target of anti-cancer anthracycline drugs, Biochem. Biophys. Res. Commun. 378 (3) (2009) 450–455.
[16] O.M. Abo-Salem, The protective effect of aminoguanidine on doxorubicin-induced nephropathy in rats, J. Biochem Mol. Toxicol. 26 (1) (2012) 1–9.
[17] M.A. Abou El Hassan, et al., The new cardioprotector Monohydroxyethylrutoside protects against doxorubicin-induced inflammatory effects in vitro, Br. J. Cancer 89 (2) (2003) 357–362.
[18] A.M. Elsharkawy, D.A. Mann, Nuclear factor-kappaB and the hepatic inflammation-fibrosis-cancer axis. Hepatology 46 (2) (2007) 590–597.
[19] F. Imam, et al., Apremilast prevent doxorubicin-induced apoptosis and inflammation in heart through inhibition of oxidative stress mediated activation of NF-κB signaling pathways, Pharmacol. Rep. 70 (5) (2018) 993–1000.
[20] M.U. Rehman, et al., D-limonene suppresses doxorubicin-induced oxidative stress and inflammation via repression of COX-2, iNOS, and NFkappaB in kidneys of Wistar rats, Exp. Biol. Med (Maywood) 239 (4) (2014) 465–476.
[21] S. Rashid, et al., Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats, Toxicol. Mech. Methods 23 (5) (2013) 337–345.
[22] S. Bouabdallah, I. Brinza, The Effect of a Tribulus-Based Formulation in Alleviating Cholinergic System Impairment and Scopolamine-Induced Memory Loss in Zebrafish (Danio rerio): Insights from Molecular Docking and In Vitro/In Vivo Approaches 17 (2) (2024).
[23] Mar Drugs.
[24] S. Ibrahim, et al., Development and Evaluation of Crocetin-Functionalized Pegylated Magnetite Nanoparticles for Hepatocellular Carcinoma 28 (7) (2023).
[25] J.A. Ross, C.M. Kasum, Dietary flavonoids: bioavailability, metabolic effects, and safety, Annu Rev. Nutr. 22 (2002) 19–34.
[26] S.H. Gerges, et al., Pharmacology of Diosmin, a Citrus Flavone Glycoside: An Updated Review, Eur. J. Drug Metab. Pharm. 47 (1) (2022) 1–18.
[27] M.S. Agir, G. Eraslan, The effect of diosmin against liver damage caused by cadmium in rats, J. Food Biochem 43 (9) (2019) e12966.
[28] A.S. Shalkami, M. Hassan, A.G. Bakr, Anti-inflammatory, antioxidant and anti-apoptotic activity of diosmin in acetic acid-induced ulcerative colitis, Hum. Exp. Toxicol. 37 (1) (2018) 78–86.
[29] S. Bouabdallah, M.H. Ibrahim, Anxiolytic and Antidepressant Effects of Tribulus terrestris Ethanolic Extract in Scopolamine-Induced Amnesia in Zebrafish: Supported by Molecular Docking Investigation Targeting Monoamine Oxidase A 17 (9) (2024).
[30] M.E.C. Bruno, et al., Accumulation of gammadelta T cells in visceral fat with aging promotes chronic inflammation, Geroscience (2022).
[31] M.M. Abdel-Daim, et al., Diosmin Attenuates Methotrexate-Induced Hepatic, Renal, and Cardiac Injury: A Biochemical and Histopathological Study in Mice, Oxid. Med Cell Longev. 2017 (2017) 3281670.
[32] D.R. Nelson, et al., Molecular Mechanisms behind Safranal's Toxicity to HepG2 Cells from Dual Omics 11 (6) (2022).

[33] G.P. Littarru, L. Tiano, Bioenergetic and antioxidant properties of coenzyme Q10: recent developments, Mol. Biotechnol. 37 (1) (2007) 31–37.
[34] K.A. Conklin, Coenzyme q10 for prevention of anthracycline-induced cardiotoxicity, Integr. Cancer Ther. 4 (2) (2005) 110–130.
[35] A.A. El-Sheikh, et al., Effect of coenzyme-Q10 on doxorubicin-induced nephrotoxicity in rats, Adv. Pharmacol. Sci. 2012 (2012).
[36] E. Sutken, et al., Protective role of melatonin and coenzyme Q10 in ochratoxin A toxicity in rat liver and kidney, Int J. Toxicol. 26 (1) (2007) 81–87.
[37] J.M. Villalba, et al., Therapeutic use of coenzyme Q10 and coenzyme Q10-related compounds and formulations, Expert Opin. Investig. Drugs 19 (4) (2010) 535–554.
[38] M. Abu Gazia, M.A. El-Magd, Ameliorative Effect of Cardamom Aqueous Extract on Doxorubicin-Induced Cardiotoxicity in Rats, Cells Tissues Organs 206 (1-2) (2018) 62–72.
[39] O.A. Alshabanah, et al., Doxorubicin toxicity can be ameliorated during antioxidant L-carnitine supplementation, Oxid. Med. Cell. Longev. 3 (6) (2010) 428–433.
[40] A.F. AlAsmari, et al., Diosmin Alleviates Doxorubicin-Induced Liver Injury via Modulation of Oxidative Stress-Mediated Hepatic Inflammation and Apoptosis via NfkB and MAPK Pathway: A Preclinical Study, Antioxid. (Basel) 10 (12) (2021).
[41] A.A. Fouad, et al., Coenzyme Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice, Toxicology 274 (1-3) (2010) 49–56.
[42] M.B. Struck, et al., Effect of a short-term fast on ketamine-xylazine anesthesia in rats, J. Am. Assoc. Lab Anim. Sci. 50 (3) (2011) 344–348.
[43] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors, Adv. Enzym. Regul. 22 (1984) 27–55.
[44] T.C. Chou, Drug combination studies and their synergy quantification using the Chou-Talalay method, Cancer Res 70 (2) (2010) 440–446.
[45] T. Hu, et al., Synergistic cardioprotective effects of Danshensu and hydroxysafflor yellow A against myocardial ischemia-reperfusion injury are mediated through the Akt/Nrf2/HO-1 pathway, Int J. Mol. Med 38 (1) (2016) 83–94.
[46] J. Lee, D. Park, Y. Lee, Metformin Synergistically Potentiates the Antitumor Effects of Imatinib in Colorectal Cancer Cells, Dev. Reprod. 21 (2) (2017) 139–150.
[47] J. Hu, et al., Synergistic induction of apoptosis in multiple myeloma cells by bortezomib and hypoxia-activated prodrug TH-302, in vivo and in vitro, Mol. Cancer Ther. 12 (9) (2013) 1763–1773.
[48] S. Rivankar, An overview of doxorubicin formulations in cancer therapy, J. Cancer Res Ther. 10 (4) (2014) 853–858.
[49] M.M. Sayed-Ahmed, et al., Inhibition of gene expression of heart fatty acid binding protein and organic cation/carnitine transporter in doxorubicin cardiomyopathic rat model, Eur. J. Pharm. 640 (1-3) (2010) 143–149.
[50] E.D.B. Danz, et al., Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway, Free Radic. Biol. Med. 46 (12) (2009) 1589–1597.
[51] X. Chen, et al., Protective effect of berberine on doxorubicininduced acute hepatorenal toxicity in rats, Mol. Med Rep. 13 (5) (2016) 3953–3960.
[52] E.Y. Podyacheva, et al., Analysis of Models of Doxorubicin-Induced Cardiomyopathy in Rats and Mice. A Modern View From the Perspective of the Pathophysiologist and the Clinician, Front Pharm. 12 (2021) 670479.
[53] P.A. Henriksen, Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention, Heart 104 (12) (2018) 971–977.
[54] S.M. Swain, F.S. Whaley, M.S. Ewer, Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials, Cancer 97 (11) (2003) 2869–2879.
[55] Z. Tian, et al., High cumulative doxorubicin dose for advanced soft tissue sarcoma, BMC Cancer 20 (1) (2020) 1139.
[56] Z. Tian, et al., Retrospective review of the activity and safety of apatinib and anlotinib in patients with advanced osteosarcoma and soft tissue sarcoma, Invest N. Drugs 38 (5) (2020) 1559–1569.
[57] N. Ali, et al., Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach, Chem. Biol. Inter. 272 (2017) 80–91.
[58] P.L. Prasanna, K. Renu, A. Valsala Gopalakrishnan, New molecular and biochemical insights of doxorubicin-induced hepatotoxicity, Life Sci. 250 (2020) 117599.
[59] D. Saleh, et al., Omega-3 fatty acids ameliorate doxorubicin-induced cardiorenal toxicity: In-vivo regulation of oxidative stress, apoptosis and renal Nox4, and in-vitro preservation of the cytotoxic efficacy, Plos One 15 (11) (2020) e0242175.
[60] M.A. Mansour, H.A. El-Kashef, O.A. Al-Shabanah, Effect of captopril on doxorubicin-induced nephrotoxicity in normal rats, Pharm. Res 39 (3) (1999) 233–237.
[61] M. Al-Shamsi, A. Amin, E. Adeghate, Vitamin E ameliorates some biochemical parameters in normal and diabetic rats, Ann. N. Y Acad. Sci. 1084 (2006) 411–431.
[62] T.A. Gonwa, H.M. Wadei, Kidney disease in the setting of liver failure: core curriculum 2013, Am. J. Kidney Dis. 62 (6) (2013) 1198–1212.
[63] S.P. Roche, R. Kobos, Jaundice in the adult patient, Am. Fam. Physician 69 (2) (2004) 299–304.
[64] M.A. Hussain, et al., Antioxidant and Anti-Inflammatory Effects of Crocin Ameliorate Doxorubicin-Induced Nephrotoxicity in Rats, Oxid. Med Cell Longev. 2021 (2021) 8841726.
[65] F. Tulubas, et al., The protective effects of omega-3 fatty acids on doxorubicin-induced hepatotoxicity and nephrotoxicity in rats, Toxicol. Ind. Health 31 (7) (2015) 638–644.
[66] N. Ali, et al., Protective effect of diosmin against doxorubicin-induced nephrotoxicity, Saudi J. Biol. Sci. 28 (8) (2021) 4375–4383.
[67] A.A. El-Sheikh, et al., Effect of coenzyme-q10 on Doxorubicin-induced nephrotoxicity in rats, Adv. Pharm. Sci. 2012 (2012) 981461.
[68] S.R. Botros, et al., Comparative effects of incretin-based therapy on doxorubicin-induced nephrotoxicity in rats: the role of SIRT1/Nrf2/NF-κB/TNF-α signaling pathways, Front Pharm. 15 (2024) 1353029.
[69] A. Khames, et al., Nicorandil combats doxorubicin-induced nephrotoxicity via amendment of TLR4/P38 MAPK/NFkappa-B signaling pathway, Chem. Biol. Inter. 311 (2019) 108777.
[70] M. Rajasekaran, Nephroprotective effect of Costus pictus extract against doxorubicin-induced toxicity on Wistar rat, ||| Bangladesh J. Pharmacol. 14 (2) (2019) 93–100.
[71] S.T. Jasim, H.M. Al-Kuraishy, A.I. Al-Gareeb, Gingko Biloba protects cardiomyocytes against acute doxorubicin induced cardiotoxicity by suppressing oxidative stress, J. Pak. Med Assoc. 69 (3) (2019) S103–S107, 8.
[72] J.B. Owen, D.A. Butterfield, Measurement of oxidized/reduced glutathione ratio, Methods Mol. Biol. 648 (2010) 269–277.
[73] A.E. Elhelaly, et al., Protective effects of hesperidin and diosmin against acrylamide-induced liver, kidney, and brain oxidative damage in rats, Environ. Sci. Pollut. Res Int 26 (34) (2019) 35151–35162.
[74] J. Lance, et al., Coenzyme Q10–a therapeutic agent, Medsurg Nurs. 21 (6) (2012) 367–371.
[75] F.M. Sohet, N.M. Delzenne, Is there a place for coenzyme Q in the management of metabolic disorders associated with obesity? Nutr. Rev. 70 (11) (2012) 631–641.
[76] T. Blatt, G.P. Littarru, Biochemical rationale and experimental data on the antiaging properties of CoQ10 at skin level, Biofactors 37 (5) (2011) 381–385.
[77] H.N. Mustafa, et al., Protective role of CoQ10 or L-carnitine on the integrity of the myocardium in doxorubicin induced toxicity, Tissue Cell 49 (3) (2017) 410–426.
[78] D.O. Saleh, R.F. Ahmed, M.M. Amin, Modulatory role of Co-enzyme Q10 on methionine and choline deficient diet-induced non-alcoholic steatohepatitis (NASH) in albino rats, Appl. Physiol., Nutr., Metab. 42 (3) (2016) 243–249.
[79] V.W. Lee, D.C. Harris, Adriamycin nephropathy: a model of focal segmental glomerulosclerosis, Nephrol. (Carlton) 16 (1) (2011) 30–38.
[80] T. Afsar, et al., Doxorubicin-induced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; Improvement by Acacia hydaspica tannin-rich ethyl acetate fraction, Saudi J. Biol. Sci. 27 (9) (2020) 2251–2260.
[81] L. Chen, et al., Inflammatory responses and inflammation-associated diseases in organs, Oncotarget 9 (6) (2018) 7204–7218.
[82] N. Fujiwara, K. Kobayashi, Macrophages in inflammation, Curr. Drug Targets Inflamm. Allergy 4 (3) (2005) 281–286.
[83] A. Soltani Hekmat, et al., Protective effect of alamandine on doxorubicininduced nephrotoxicity in rats, BMC Pharm. Toxicol. 22 (1) (2021) 31.
[84] S. Song, et al., Protective Effects of Dioscin Against Doxorubicin-Induced Hepatotoxicity Via Regulation of Sirt1/FOXO1/NF-kappab Signal, Front Pharm. 10 (2019) 1030.
[85] Y. Zhang, et al., Protective effects of dioscin against doxorubicin-induced nephrotoxicity via adjusting FXR-mediated oxidative stress and inflammation, Toxicology 378 (2017) 53–64.
[86] Y. Abdalla, et al., Safranal Prevents Liver Cancer Through Inhibiting Oxidative Stress and Alleviating Inflammation, Front Pharm. 12 (2021) 777500.
[87] M.M. Anwar, et al., Impact of Lyophilized Milk Kefir-Based Self-Nanoemulsifying System on Cognitive Enhancement via the Microbiota–Gut–Brain Axis, Antioxidants 13 (10) (2024) 1205.
[88] S. Abdu, N. Juaid, A. Amin, Ther. Eff. Crocin Alone Or. Comb. Sorafenib Hepatocell. Carcinoma.: Vivo Vitr. Insights 11 (9)) (2022).
[89] A.F. Wali, et al., Naringenin Regulates Doxorubicin-Induced Liver Dysfunction: Impact on Oxidative Stress and Inflammation, Plants (Basel) 9 (4) (2020).
[90] A. Kalantary-Charvadeh, et al., Micheliolide Protects Against Doxorubicin-Induced Cardiotoxicity in Mice by Regulating PI3K/Akt/NF-kB Signaling Pathway, Cardiovasc Toxicol. 19 (4) (2019) 297–305.
[91] M.A. Ali, et al., Caffeic acid phenethyl ester counteracts doxorubicin-induced chemobrain in Sprague-Dawley rats: Emphasis on the modulation of oxidative stress and neuroinflammation, Neuropharmacology 181 (2020) 108334.
[92] A.O. S Yousef, et al., The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities, Int. J. Environ. Res. Public Health 16 (16) (2019) 2895.
[93] W.A. Al-Megrin, et al., Coenzyme Q10 Activates the Antioxidant Machinery and Inhibits the Inflammatory and Apoptotic Cascades Against Lead Acetate-Induced Renal Injury in Rats, Front Physiol. 11 (2020) 64.
[94] A. Liskova, et al., Flavonoids as an effective sensitizer for anti-cancer therapy: Insights into multi-faceted mechanisms and applicability towards individualized patient profiles, EPMA J. 12 (2) (2021) 155–176.
[95] H. Greenlee, et al., Lack of effect of coenzyme q10 on doxorubicin cytotoxicity in breast cancer cell cultures, Integr. Cancer Ther. 11 (3) (2012) 243–250.
[96] N.K. Swarnakar, K. Thanki, S. Jain, Effect of co-administration of CoQ10-loaded nanoparticles on the efficacy and cardiotoxicity of doxorubicin-loaded nanoparticles, RSC Adv. 3 (34) (2013) 14671–14685.