[0] www.researchgate.net/publication/311583509_Possible_involvement_of_Nrf2_and_PPARg_up-regulation_in_the_protective_effect_of_umbelliferone_ag
2.9% 35 matches

[1] www.researchgate.net/publication/349723796_Protective_effect_of_pravastatin_on_doxorubicin-induced_hepatotoxicity
2.3% 36 matches

[2] www.ncbi.nlm.nih.gov/pmc/articles/PMC6920116/
3.7% 15 matches

[3] www.ncbi.nlm.nih.gov/pmc/articles/PMC8083003/
2.2% 31 matches

[4] www.mdpi.com/2218-273X/9/11/703/htm
0.8% 33 matches

[5] www.mdpi.com/2223-7747/9/4/550/htm
0.3% 29 matches

[8] www.researchgate.net/publication/318975397_Allicin_ameliorates_doxorubicin-induced_cardiotoxicity_in_rats_via_suppression_of_oxidative_stress_infl
1.5% 20 matches

[9] www.researchgate.net/figure/Histopathological-examination-of-liver-tissue-showing-a-section-from-normal-control-rats_fig9_326378958
3.2% 8 matches

[10] www.frontiersin.org/articles/484821
0.1% 25 matches

[13] www.karger.com/Article/FullText/475639
0.8% 21 matches

[16] 18β-Glycyrrhetinic acid protects against methotrexate-induced kidney injury by up-regulating the Nrf2/ARE/HO-1 pathway and endogenous antioxidants
0.5% 22 matches

[17] www.researchgate.net/figure/A-Control-group-showing-normal-hepatocytes-preserved-architecture-and-liver-cells_fig5_325666664
2.5% 7 matches

[18] www.epj.eg.net/viewimage.asp?img=EgyptPharmaceutJ_2019_18_3_208_266661_f5.jpg
2.4% 7 matches

[19] www.mdpi.com/2076-2615/11/3/886/pdf
0.4% 17 matches

[20] Gamma-Glutamylcysteine Ethyl Ester Protects against Cyclophosphamide-Induced Liver Injury and Hematologic Alterations via Upregulation of PPAR[
0.0% 25 matches

[21] www.researchgate.net/publication/329621635_Doxazosin_and_Carvedilol_Treatment_Improves_Hepatic_Regeneration_in_a_Hamster_Model_of_Cirrhos
0.1% 21 matches

[23] journals.plos.org/plosone/article?id=10.1371/journal.pone.0064526
0.6% 17 matches

[24] Hepatoprotective effect of Commiphora myrrha against d-GalN/LPS-induced hepatic injury in a rat model through attenuation of pro inflammatory cyto
0.6% 18 matches

[25] from a PlagScan document dated 2017-07-11 10:39
0.3% 20 matches

[26] www.researchgate.net/publication/303910842_Protective_Effects_of_Ruitn_and_or_Hesperidin_Against_Doxorubicin-Induced_Hepatotoxicity
0.2% 18 matches

[27] www.researchgate.net/publication/24238986_Liposome_encapsulation_of_curcumin_and_resveratrol_in_combination_reduces_prostate_cancer_incidence
0.3% 14 matches

[28] www.mdpi.com/1422-0067/21/6/2048/pdf
0.2% 17 matches

[30] centaur.reading.ac.uk/76171/2/22828005_Giallourou_thesis.pdf
0.4% 12 matches

[31] www.researchgate.net/publication/333092480_Protective_effect_of_morin_on_doxorubicin-induced_hepatorenal_toxicity_in_rats
0.3% 9 matches

[34] Protective Effects of Taraxasterol against Ethanol-Induced Liver Injury by Regulating CYP2E1/Nrf2/HO-1 and NF-[kappa]B Signaling Pathways in Mi
0.2% 14 matches

[35] from a PlagScan document dated 2018-03-20 09:11

0.3% 11 matches

[37] "Zaid4.pdf" dated 2016-12-22
0.6% 9 matches

[38] Inhibition of NF-κB/TNF-α pathway may be involved in the protective effect of resveratrol against cyclophosphamide-induced multi-organ toxicity
0.0% 16 matches

[39] www.nature.com/articles/s41598-020-71827-9
0.4% 10 matches

[41] Effects of erdosteine on cyclosporine-A–induced hepatotoxicity in rats
0.0% 12 matches

[42] www.researchgate.net/figure/Light-microscopy-of-kidney-in-diabetic-MSCs-group-showed-congestion-C-in-glomerular_fig12_307744431
0.2% 13 matches

[44] www.spandidos-publications.com/10.3892/ijmm.2017.3282
0.4% 11 matches

[45] from a PlagScan document dated 2017-12-07 21:34
0.5% 10 matches

[46] www.tandfonline.com/doi/full/10.3109/0886022X.2016.1165120
0.2% 12 matches

[47] from a PlagScan document dated 2018-03-06 20:42
0.0% 14 matches

[48] Protective effects of sesamin on liver fibrosis through antioxidative and anti-inflammatory activities in rats
0.2% 11 matches

[49] Differential effects of eugenol against hepatic inflammation and overall damage induced by ischemia/re-perfusion injury
0.3% 13 matches

[51] from a PlagScan document dated 2016-03-27 10:59
0.4% 9 matches

[53] www.oatext.com/the-potential-protective-role-of-grape-seed-proanthocyanidin-extract-against-the-mixture-of-carboplatin-and-thalidomide-induced-hepat
0.1% 10 matches

[54] www.frontiersin.org/articles/10.3389/fphar.2019.01081/full
0.2% 12 matches

[56] www.researchgate.net/publication/324996332_Cardioprotective_effect_of_zingerone_against_oxidative_stress_inflammation_and_apoptosis_induced_by_
0.2% 9 matches
⊞ 1 documents with identical matches

[59] from a PlagScan document dated 2018-06-11 21:11
0.0% 11 matches

[60] from a PlagScan document dated 2020-08-10 17:44
0.0% 11 matches

[61] "يزيد عبدالله الشويشي.pdf" dated 2021-03-25
0.4% 8 matches

[62] from a PlagScan document dated 2020-04-21 12:39
0.0% 11 matches

[63] from a PlagScan document dated 2018-01-29 07:08
0.4% 6 matches

[64] journals.physiology.org/doi/pdf/10.1152/ajpcell.00356.2016
0.0% 8 matches

[65] "Manu. without.docx" dated 2020-07-21
0.2% 10 matches
⊞ 1 documents with identical matches

[67] from a PlagScan document dated 2020-07-13 04:42
0.1% 9 matches

[68] from a PlagScan document dated 2021-03-31 08:43
0.2% 11 matches

[69] www.hindawi.com/journals/bmri/2020/8593617/
0.0% 8 matches

[72] www.ncbi.nlm.nih.gov/pmc/articles/PMC7877852/
0.2% 7 matches

[74] journals.physiology.org/doi/10.1152/ajpcell.2001.280.3.C659
0.0% 8 matches

☑	[75]	from a PlagScan document dated 2016-06-29 08:55 0.0% 8 matches
☑	[77]	from a PlagScan document dated 2020-02-02 14:29 0.0% 8 matches
☑	[78]	from a PlagScan document dated 2019-11-23 18:14 0.1% 5 matches
☑	[81]	www.frontiersin.org/articles/10.3389/fphar.2014.00025/full 0.2% 6 matches
☑	[82]	"Manuscript 1 (1).docx" dated 2020-07-13 0.0% 7 matches
☑	[84]	"The correlation between single nucleotide polymorphisms of the thymic stromal lymphopoietin receptor and breast cancer.pdf" dated 2021-07-26 0.0% 7 matches
☑	[86]	"22015.pdf" dated 2017-10-30 0.2% 6 matches
☑	[87]	article.sciencepublishinggroup.com/html/10.11648.j.ijbmr.20150304.11.html 0.3% 6 matches
☑	[88]	www.researchgate.net/figure/The-level-of-pro-anti-inflammatory-cytokines-in-liver-homogenate-from-intact-and_tbl1_26697062 0.3% 4 matches
☑	[89]	"Nouf's Thesis-edited- Farooq-similarity check.docx" dated 2021-03-16 0.2% 6 matches
☑	[91]	journals.sagepub.com/doi/pdf/10.1177/014556131609501211 0.0% 9 matches
☑	[92]	www.researchgate.net/publication/350461962_Xinmailong_Attenuates_Doxorubicin-Induced_Lysosomal_Dysfunction_and_Oxidative_Stress_in_H9c2_C 0.2% 6 matches
☑	[94]	from a PlagScan document dated 2020-07-09 10:28 0.0% 8 matches
☑	[95]	from a PlagScan document dated 2018-12-28 13:03 0.3% 4 matches
☑	[96]	www.ncbi.nlm.nih.gov/pmc/articles/PMC8290492/ 0.1% 5 matches
☑	[97]	from a PlagScan document dated 2017-11-21 10:12 0.2% 4 matches
☑	[98]	www.researchgate.net/publication/321266993_Synergistic_Anticancer_Effect_of_Paclitaxel_and_Noscapine_on_Human_Prostate_Cancer_Cell_Lines 0.0% 6 matches
☑	[99]	"vms3.345.pdf" dated 2020-09-10 0.0% 5 matches
☑	[100]	from a PlagScan document dated 2019-01-22 20:05 0.0% 5 matches
☑	[101]	www.frontiersin.org/articles/513342 0.0% 6 matches

14 pages, 5074 words

⚠ A very light text-color was detected that might conceal letters used to merge words.
⚠ The document contains a suspicious mixture of alphabets. This could be an attempt of cheating.

PlagLevel: 13.2% selected / 31.3% overall

192 matches from 104 sources, of which 51 are online sources.

Settings

Data policy: *Compare with web sources, Check against organization repository, Check against the Plagiarism Prevention Pool*

Sensitivity: *High*

Bibliography: *Bibliography excluded*

Citation detection: *Highlighting only*

Whitelist: --

The beneficial efficacy of liposomal resveratrol against doxorubicin-induced hepatotoxicity in rats: Role of TGF-β1 and SIRT1

Ahlam M. Alhusaini[1], Abeer M. Alanazi[1], Laila M. Fadda[1], Qamraa H. Alqahtani[1], Wedad S. Sarawi[1] and Iman H Hasan[1]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University.

Abstract

Objectives: Doxorubicin (DOXR) belongs to the antineoplastic anthracycline antibiotic and despite its prevalent use for various types of cancer, it is associated with side effects that occur even after treatment cessation, particularly on the heart, blood, liver and kidney. This study aimed to study the efficiency of carvedilol (CAR), resveratrol (RES) or liposomal RES (LIPO-RES) and their combination to treat DOXR-induced hepatotoxicity and to investigate other driving mechanisms that seem to be involved.

Methods: Rats were injected twice weekly with DOXR for five weeks to induce liver injury. A week before DOXR injection commenced, rats were pretreated singly or simultaneously with CAR, RES or LIPO-RES for six weeks.

Results: A significant elevation in serum alanine aminotransferase (ALT) and changed the hepatic tissue structure following DOXR administration indicating hepatic injury. Additionally, DOXR injection caused upregulation of the hepatic malondialdehyde (MDA), inflammatory cytokines, and transforming growth factor-β1 (TGF-β1) levels. The endogenous glutathione (GSH) and sirtuin1 (SIRT1) expressions in the liver were downregulated following DOXR administration. CAR, RES or LIPO-RES as their alternative combinations attenuated the liver injury by controlling oxidative stress, inflammation, and fibrosis. These beneficial effects were apparent upon using combined CAR and LIPO-RES, as proved by the restoring the balance of MDA and GSH levels, decreasing hepatic cytokines levels of IL-6 and TNF-α, upregulating SIRT1 and downregulating TGF-β1 levels.

Conclusion: the current study conferred that the use of CAR, RES or LIPO-RES alone or combination could prevent DOXR-induced hepatic damage in rats by inhibiting oxidative stress, inflammation, and fibrosis.

Keywords

hepatotoxicity, TGF-β1, SIRT1, carvedilol, doxorubicin, resveratrol.

1. Introduction

Doxorubicin (DOXR) represents one of the commonly used chemotherapeutic agents in many types of malignancy that mainly inhibit DNA synthesis. Apart from its high antitumor activity, DOXR has been linked to many adverse effects, especially cardiotoxicity and hepatotoxicity.[21] The hepatotoxicity that is associated with DOXR occurs in the form of hepatocyte degeneration and hyperplasia of the bile duct together with focal necrosis [1].[87] The major mechanism thought to be implicated in DOXR-induced hepatotoxicity is the overproduction of reactive oxygen species (ROS) as a consequence of its hepatic metabolism, which leads to oxidative stress.[30] The induction of ROS production is linked to p53 activation and results in elevation of Bax and Fas expressions which in turn induces the expression of caspase-3 and eventually elicits apoptotic signaling [2].[0] In addition, DOXR is associated with the initiation of inflammatory response in the liver through oxidative stress by upregulation of the mRNA expression of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and connective tissue growth factor (CTGF) which can be involved in TGF-β1 downstream actions [3]. DOXR causes activation of nuclear factor-kappa B (NF-κB) signaling through the inhibition of sirtuin 1 (SIRT1) and this results in inflammatory reaction [4]. Moreover, DOXR disrupts the mitochondrial function through the ROS production as it enters the mitochondrial matrix with a high affinity to its inner membrane [5]. Mitochondrial dysfunction is attributed either to the reduction of inorganic phosphate and thereby decreasing adenosine triphosphate (ATP) level, or inhibition of mitochondrial DNA replication [5].

Based on the molecular mechanism of DOXR-hepatotoxicity, the use of antioxidant and anti-inflammatory agents can effectively improve the hepatic complications elicited by this chemotherapeutic agent. Resveratrol (RES) is a natural polyphenolic compound that possesses antioxidant, anti-inflammatory and anti-aging properties present largely in some edible plants like grapes [6]. Similarly, carvedilol (CAR) is an agent that belongs to third generation β-blocker which inhibits β-1, β-2 and α-1 adrenoceptors and has an antioxidant effect that may exert additional beneficial action to protect against DOXR-induced hepatotoxicity [7]. In cardiomyocytes, CAR inhibits lipid peroxidation and protects them from deleterious effects of oxidative stress [8]. Likewise, it inhibits activated neutrophils from releasing superoxide anion

[9]. Moreover, CAR was shown to protected cultured endothelial cells against cell injury caused by ROS [10].[45]

As mentioned earlier, DOXR therapy is associated with harmful effects on liver, and unfortunately, there are limited treatment options to overcome this limitation.[45] Therefore, there is an urgent need for the emergence of new and advanced strategies for controlling DOXR's hepatic side effects. Liposomal form of RES is considered as one of these promising protective approaches. This form of RES can increase drug bioavailability, decrease immune reactivity, and target organs with the specific drug. This study aims to evaluate the beneficial activities of RES, CAR or liposomal analog of RES against the DOXR-induced liver injury in rats, focusing on their antioxidative, anti-inflammatory, and antifibrotic actions.

2. Materials and Methods

2.1. Chemicals

RES and CAR were obtained from Sigma (St. Louis, USA). DOXR (Ebewe Pharma Co, Unterach Am Attersee, Austria) was kindly provided from a local pharmacy in Riyadh (Saudi Arabia), and the liposomal Trans RES® formulation (RES encapsulated in liposomes with particle size = 200 nm) was obtained from Lipolife® (Drakes Lane, UK). Primary antibodies for TGF-β1 (ab92486), SIRT1 (ab7343) and GAPDH (ab9483) were obtained from Abcam® (Cambridge, USA).[44] The secondary antibodies conjugated to horseradish peroxidase (HRP) (sc-2357 and sc-2354) were purchased from Santa Cruz Biotechnology (Dallas, USA).

2.2.[3] Animal and experimental design

Male Wistar albino rats of an average weight of 165±10 g were obtained from the Animals Care Centre at the College of Pharmacy, King Saud University, Riyadh, Saudi, KSA.[37] Prior to the experiment, the rats were allowed to adapt to the laboratory conditions (adjusted temperature at 23 ºC with alternation of 12/12 h light/dark cycle) for at least one week with free access to food and water ad libitum.[3] All experiments were conducted according to the guidelines and regulations of the Research Ethics Committee at King Saud University (Ref. No: KSU-SE-18-31).

[0] A total of forty-two rats were distributed into seven groups, with six rats each as follows:

Group I (Control): received 1% carboxymethylcellulose (CMC) orally which was used as the vehicle of the drugs for 6 weeks. Physiological saline was given intraperitoneally (i.p.) to this group, starting from week 2 to week 6 twice every week.

Group II (DOXR): received 1% CMC orally for 6 weeks and the DOXR treatment was given twice every week (2 mg/kg, i.p.) starting from week 2 to week 6 to give rise to a total cumulative dose of 20 mg/kg [11].

Group III (CAR): treated with CAR (30 mg/kg) orally for 6 weeks and DOXR treatment was given from the second week as referred in group II [12].

Group IV (RES): treated with RES (20 mg/kg) orally for 6 weeks and DOXR treatment was given from the second week as referred in group II [13].

Group V (CAR/RES): received a combination of CAR (30 mg/kg) and RES (20 mg/kg) orally for 6 weeks and DOXR treatment was given from the second week as referred in group II.

Group VI (LIPO-RES): treated with LIPO-RES (20 mg/kg) orally for 6 weeks and DOXR treatment was given from the second week as referred in group II.

Group VII (CAR/LIPO-RES): received a combination of CAR (30 mg/kg) and LIPO-RES (20 mg/kg) orally for 6 weeks and DOXR treatment was given from the second week as referred in group II.

After completion of treatment courses, all rats were anesthetized, and blood samples were collected and centrifuged to collect serum. Then, the livers were collected from rats and washed. Parts from the livers were fixed in 10% formalin for histopathological examination, whereas the remaining parts were homogenized in chilled phosphate buffered saline (PBS) and proteinase inhibitors and then centrifuged. The supernatants were kept at -80°C for the subsequent experiments.

2.3. Assay of liver function, cytokines levels and oxidative stress markers

Serum alanine aminotransferase (ALT) was measured employing a kit obtained from Randox (Crumlin, UK). Hepatic tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were assayed using ELISA kits (MyBioSource, CA, USA) as instructed by the manufacturer. Hepatic level of malondialdehyde (MDA), a well-known indicator of lipid peroxidation, was

assayed as before described.[5] Briefly, the homogenate tissue was mixed with TBA, and acetate buffer, and then heated in boiling water bath for one hour.[35] Once cooling, n-butanol was added to the mixture, which was centrifuged, the absorbance was measured at 532 nm [14]. Hepatic GSH level was determined according to the method of Ellman;[35] this analysis is based on the reaction of GSH with 5,50-dithio-bis (2-nitrobenzoic acid) and measurement of the absorbance at 412 nm [15].

2.4. DNA fragmentation

The level of DNA fragmentation in hepatic cells as an indicator for cellular death was analyzed by detecting the labeled DNA fragments in the samples using a DNA fragmentation kit (Cat no. 6137, Takara Bio, USA).

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

According to kit instructions, frozen liver tissues were used to extract total RNA by RNeasy Mini Kit (Qiagen, Cat. No. 74104).[16] The reverse transcription step was performed to synthesize the complementary DNA (cDNA) by First Strand cDNA Synthesis Kit (Cat. No. NP100041). Primers for heme oxygenase-1 (HO-1), Ferritin and β-actin genes are listed in Table 1. After that, the primers were mixed with cDNA, Platinum Blue PCR SuperMix (Invitrogen) and nuclease-free water in PCR tubes. The cycling reactions were performed using Biorad T100 thermal cycler. The amplified DNA samples were loaded into agarose gel mixed with ethidium bromide and allowed to run for 30 min at 120 V in Tris-acetate-EDTA buffer (TAE). The gels were visualized with the Gene Genius Bioimaging System (Syngene).

Table 1. The primer sequences for genes KP enzymes RT-PCR.

Gene	Primer sequence
HO-1	F: 5′- -3′ AGCATGTTCCCAGGATG R: 5′- -3′ GCTCAATGTTGAGCACA
Ferritin	F: 5′- -3′ GCC CTG AAG AAC TTT GCC AAA T R: 5′- -3′ TGC AGG AAG ATT CGT CCA CCT
β-actin	F: 5′- -3′ CTG TCCCTGTATGCCTCT R: 5′- -3′ ATGTCACGCACGATTTCC

2.6. Western Blotting

The expression of TGF-β1 and SIRT1 in the liver samples was estimated by Western blotting, where the hepatic cells were lysed with RIPA buffer. After protein concentration measurement, 40µg of the total protein was loaded on 12% SDS/PAGE and subjected to electrophoresis to separate the proteins based on their molecular sizes. The proteins were transferred into nitrocellulose membranes and then blocked with 5% skimmed milk. After that, the membranes were incubated with either anti-TGF-β1, anti-SIRT1 or anti-GAPDH for overnight.[23] Then, the membranes were washed several times and probed with secondary antibodies for 2 hours. Finally, the membranes were washed and imaged in ImageQuant LAS 4000 (GE Healthcare, USA) using ECL chemiluminescent detection kit (Bio-Rad, USA). The protein bands were scanned and quantified using ImageJ (NIH, USA).

2.7. Histological studies

Liver samples that were fixed in 10% neutral buffered formalin, then, after dehydration, they had been inlaid on paraffin wax. The livers were cut into 5-µm-thick sections µm, then, stained with hematoxylin and eosin (H&E) after deparaffinization. The sections were dehydrated and examined under a light microscope.

2.8.[96] Statistical Analysis

The data were presented as mean ± standard error of the mean (SEM).[54] Significant differences between the study groups were measured by employing one-way ANOVA followed by Tukey's post-hoc test using GraphPad 7 software (GraphPad, USA) and considering P-value of 0.05 as statistically significant.

3. Results

3.1.[0] DOXR-induced hepatotoxicity, oxidative stress and cell damage were ameliorated by RES, CAR and LIPO-RES

To investigate the possible protective effects of RES, LIPO-RES and/or CAR on DOXR-induced hepatotoxicity, we measured serum liver enzyme ALT, hepatic DNA fragmentation. DOXR elicited a significant elevation of ALT serum level, which restored to the normal level after treatments (Figure 1).[0] In addition, to assess the effect of earlier mentioned treatment on

DOXR-induced oxidative stress on liver, we measured MDA and GSH levels. In DOXR-intoxicated group, there were significant elevation in MDA level and reduction in GSH level; However, these deleterious changes in the oxidative stress markers were ameliorated after administration of CAR, RES or LIPO-RES profoundly decrease MDA and increase GSH levels (Figure 2). Also, to evaluate the impact of current treatments on hepatic cellular death, we measured the DNA fragmentation in hepatic tissue samples. As expected, the DOXR-intoxicated group showed a significant DNA fragmentation level compared to the control group (p 0.001). On the contrary, treatment with CAR, RES or LIPO-RES individually or in combination resulted in a significant reduction of DOXR-induced DNA fragmentation (p 0.001) (Figure 3).

3.2. CAR, RES and LIPO-RES reduced the inflammatory markers levels:

The levels of TNF-α and IL-6 were significantly elevated in the liver of rats using DOXR; Whereas, treatment with CAR, RES or LIPO-RES individually or in combination significantly reduced the hepatic levels of both cytokines (Figure 4). Among the treatment groups, LIPO-RES containing regimen showed the stronger effect in reducing TNF-α (p 0.01). However, IL-6 level was significantly reduced in the group that received the combination of CAR and LIPO-RES (p 0.01).

3.3. CAR, RES and LIPO-RES modulated the gene expression of HO-1 and Ferritin:

The use of CAR, RES and LIPO-RES treatments increased the gene expression of HO-1 that was reduced with DOXR toxicity, this change was significant with the treatment combinations of CAR+RES and CAR+LIPO-RES (p 0.05 and p 0.001 respectively). In the contrary, the expression of ferritin gene was reduced after such combinations (p 0.05 and p 0.01 respectively), as depicted in Figure 5.

3.4. The roles of SIRT1 and TGF-β1 in DOXR-induced hepatotoxicity:

To investigate the involvements of SIRT1 and TGF-β1 in DOXR-induced hepatic abnormalities and whether the use of CAR, RES and LIPO-RES can control the expression of these proteins, Western blotting was employed. Figure 6 showed that CAR, RES and LIPO-RES treatments significantly increased SIRT1 and decreased TGF-β1 and protein expression.

3.5. CAR, RES and LIPO-RES ameliorated the hepatic histopathological changes in response to DOXR:

Histopathological studies showed that the liver section from the DOXR treated group showed congestion of sinusoids, central vein, and portal tract vein with the appearance of binucleated hepatocytes. Treatment with CAR resulted in normal structure and architecture of hepatic tissue with mild hydropic changes and congested sinusoids. RES treated group showed the same features as CAR and congested central vein, binucleated cells, and aggregates of lymphocytes at portal tract. LIPO-RES group showed almost the same effects as RES group without aggregation of lymphocytes. [2] The combination groups showed hepatic tissue with normal morphology, hepatocytes arranged in thin plates and dilated sinusoids and central vein (Figure 7).

[95]
4. Discussion

Hepatotoxicity is one of the clinical consequences associated with DOXR therapy in a significant proportion of the patients (about 30%) [16]. The exact underlying molecular mechanisms of DOXR-induced hepatotoxicity are not fully understood, and little is known about effective treatments for such adverse events. So, this study was conducted to examine the effectiveness of combining two well-known antioxidants, namely RES and CAR, in alleviating the DOXR-induced hepatotoxicity in rat model. In the current study, DOXR-induced liver damage was established by increasing ALT activity level, whereas, CAR, RES, LIPO-RES, and the combination treatments decreased the activity level of ALT enzyme. The combination of CAR and LIPO-RES treatment exerted a significant reduction in ALT level matched with the combination of CAR and RES treatment, which indicates the beneficial effect of liposomal preparations on the bioavailability of RES [17]. ALT enzyme is the most sensitive and the primary indicator for liver function, as its elevation level is associated with liver injury [4], [18].

Herein, LIPO-RES alone and the combination of CAR with either RES or LIPO-RES exhibited protective effects against DOXR-induced oxidative stress via restoring the normal levels of MDA and GSH. CAR is a potent antioxidant agent that is possessed due to its carbazole moiety [19]. Other mechanism is related to the sequestering of ferrous (Fe^{2+}) ion that inhibits lipid peroxidation [20]. Moreover, previous studies provided evidence that RES can decrease ROS production as it has free radical scavenging property [21], [22], and has the ability to inhibit

NADPH oxidase enzyme [14]. RES showed a protective effect in hepatocytes culture against oxidative stress via increasing the actions of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase [23].

DOXR-induced hepatocyte inflammation is considered an important mechanism responsible for its hepatotoxicity through stimulation of pro-inflammatory cytokines release [24], [25]. We found that treatment with LIPO-RES individually or in combination with CAR highly produced reduction in TNF-α and IL-6 concentrations. Our results agree with those reported by Kandil et al., who examined the protective effect of RES against CCl4-induced hepatic damage, and they documented that RES pre-treatment significantly reduced pro-inflammatory cytokines such as IL-6 [26]. Numerous studies revealed the anti-inflammatory property of RES, as it reduced intercellular adhesion molecule 1 (ICAM-1), inducible nitric oxide synthase (iNOS), TNF-α- and IL-6 as well as NF-κB levels [27].

In addition, DOXR toxicity is also related to its ability to down-regulate the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), a cellular transcription factor, which exerts antioxidant defense mechanisms against oxidative stress. Nrf-2 induces the transcription process of essential antioxidant enzymes such as glutathione S-transferase, NAD(P)H1 and catalase [28]. Nrf-2 also regulates the gene expression of HO-1. HO-1 is an important antioxidant, anti-inflammatory, and anti-apoptotic agent. Bai et al. reported that DOXR downregulated Nrf-2, and subsequently, the gene expressions of HO-1 [29]. Consistent with these observations, our results showed that DOXR reduced HO-1 gene expression.

Further, the importance of iron in DOXR-induced cardiac and liver toxicity is extensively recognized. Ferritin is the major iron storage protein and the enhancement of its production in case of DOXR treatment is considered a defensive mechanism to reduce the amount of iron available for ROS synthesis, and subsequently prevent oxidative stress [30]. In the present study, DOXR-induced hepatocyte damage was further manifested by the significant elevation in ferritin gene level, while these effects were prevented by the combination of CAR with either RES or LIPO-RES. The effect of RES on HO-1 was documented in previous studies, it suppressed NF-κB and stimulated Nrf2/HO-1 pathways in rats with acute liver injury [31]. Moreover, a recent study by Zhang et al. reported that the combination of CAR and carnosic acid significantly increased HO- 1 expression [32].

Our results indicated that the administration of DOXR significantly decreased SIRT1 protein expression, also it increased the DNA damage proved by DNA fragmentation result. While SIRT1 is an NAD^+- dependent deacetylase protein, it affects the metabolism of numerous target proteins in several tissues as the heart, liver, muscle, and adipose tissue.[23] SIRT1 plays a critical role in oxidative stress, and it has a protective action against many diseases, including neurologic, cancer and cardiovascular diseases [33]. Several studies have shown that the activation of SIRT1 is considered a cardioprotective strategy in cardiovascular diseases, including DOXR-induced cardiotoxicity [34]. In the present work, treatment with CAR, RES, LIPO-RES and the combination of CAR with either RES or LIPO-RES increased SIRT1 protein expression as well as decreased DNA fragmentation. Studies have shown that RES protects cardiac cells in rat animal models against myocarditis via up regulating the expression of SIRT1 that reduced apoptosis mediated by FoxO1 pathway [35].

Our data showed that DOXR administration progressed fibrosis by increasing TGF-β1 protein expression inconsistent with a prior study [36]. TGF-β1 is a key fibrogenic factor and plays a leading role in the fibrotic process in different tissues, including cardiac, renal, and hepatic tissue [37]. DOXR up-regulated TGF- β1/Smad3 pathway in DOXR-induced heart failure rats [38]. However, we found that treatment with LIPO-RES alone and the combination of CAR with either RES or LIPO-RES reduced TGF-β1 level. Interestingly, according to our research results, the highest reduction of TGF-β1 expression was achieved by the combination of CAR and LIPO-RES treated group compared with the DOXR group. A recent study by Zhang et al. reported that RES treatment significantly decreased the TGF-β1 by affecting Smad7 protein expression [39]. Furthermore, another study conducted by Serna et al. in a liver cirrhosis model found that CAR administration caused a reduction in TGF-β1 level [40]. Taken together, these results expanded our understanding of DOXR hepatotoxic mechanisms by highlighting the potential role of SIRT1 and TGF-β1 in such toxicity.

Author Contributions

Conceptualization: A.A., L.F. and A.M.A.; methodology: A.A.; L.A.; and A.M.A.; validation: A.A.; A.M.A. and I.H.H; data analysis: W.S., Q.A. and I.H.H; investigation, A.A.; L.F. W.S. and I.H.H; writing, review, and editing: A.A., A.M.A. W.S., Q.A. and I.H.H supervision, A.A.; L.F. and I.H.H.

Acknowledgment

This research project was supported by a grant from the Research Center of the Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University, Saudi Arabia.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] P. Shivakumar, M. U. Rani, A. G. Reddy, and Y. Anjaneyulu, "A study on the toxic effects of doxorubicin on the histology of certain organs," Toxicol. Int., vol. 19, no. 3, p. 241, 2012.

[2] K. Nagai, S. Fukuno, A. Oda, and H. Konishi, "Protective effects of taurine on doxorubicin-induced acute hepatotoxicity through suppression of oxidative stress and apoptotic responses," Anticancer. Drugs, vol. 27, no. 1, pp. 17–23, 2016.

[3] O. Cengiz et al., "Use of selenium to ameliorate doxorubicin induced hepatotoxicity by targeting pro-inflammatory cytokines," Biotech. Histochem., vol. 96, no. 1, pp. 67–75, 2021.

[4] S. Song et al., "Protective effects of dioscin against doxorubicin-induced hepatotoxicity via regulation of Sirt1/FOXO1/NF-κb signal," Front. Pharmacol., vol. 10, p. 1030, 2019.

[5] C. Guven, Y. Sevgiler, and E. Taskin, "Mitochondrial dysfunction associated with doxorubicin," in Mitochondrial diseases, IntechOpen, 2018, p. 323.

[6] G. Giovinazzo, I. Ingrosso, A. Paradiso, L. De Gara, and A. Santino, "Resveratrol biosynthesis: plant metabolic engineering for nutritional improvement of food," Plant foods Hum. Nutr., vol. 67, no. 3, pp. 191–199, 2012.

[7] K. V. Kumar, A. A. Shifow, M. U. R. Naidu, and K. S. Ratnakar, "Carvedilol: a beta blocker with antioxidant property protects against gentamicin-induced nephrotoxicity in rats," Life Sci., vol. 66, no. 26, pp. 2603–2611, 2000.

[8] N. Noguchi, K. Nishino, and E. Niki, "Antioxidant action of the antihypertensive drug, carvedilol, against lipid peroxidation," Biochem. Pharmacol., vol. 59, no. 9, pp. 1069–1076, 2000.

[9] T.-L. Yue, P. J. McKenna, R. R. Ruffolo Jr, and G. Feuerstein, "Carvedilol, a new β-adrenoceptor antagonist and vasodilator antihypertensive drug, inhibits superoxide release from human neutrophils," Eur. J. Pharmacol., vol. 214, no. 2–3, pp. 277–280, 1992.

[10] T.-L. Yue, X. Wang, J.-L. Gu, R. R. Ruffolo Jr, and G. Z. Feuerstein, "Carvedilol prevents low-density lipoprotein (LDL)-enhanced monocyte adhesion to endothelial cells by inhibition of LDL oxidation," Eur. J. Pharmacol., vol. 294, no. 2–3, pp. 585–591, 1995.

[11] E. Tatlidede et al., "Resveratrol treatment protects against doxorubicin-induced cardiotoxicity by alleviating oxidative damage," Free Radic. Res., vol. 43, no. 3, pp. 195–205, 2009.

[12] W. Arozal et al., "Protective effect of carvedilol on daunorubicin-induced cardiotoxicity and nephrotoxicity in rats," Toxicology, vol. 274, no. 1–3, pp. 18–26, 2010.

[13] N. Chehl, G. Chipitsyna, Q. Gong, C. J. Yeo, and H. A. Arafat, "Anti-inflammatory effects

of the Nigella sativa seed extract, thymoquinone, in pancreatic cancer cells," Hpb, vol. 11, no. 5, pp. 373–381, 2009.
[14] H. S. Shoukry et al., "Prophylactic supplementation of resveratrol is more effective than its therapeutic use against doxorubicin induced cardiotoxicity," PLoS One, vol. 12, no. 7, p. e0181535, 2017.
[15] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Anal. Biochem., vol. 95, no. 2, pp. 351–358, 1979.
[16] G. L. Ellman, "Tissue sulfhydryl groups," Arch. Biochem. Biophys., vol. 82, no. 1, pp. 70–77, 1959.
[17] G. Damodar, T. Smitha, S. Gopinath, S. Vijayakumar, and Y. A. Rao, "An evaluation of hepatotoxicity in breast cancer patients receiving injection Doxorubicin," Ann. Med. Health Sci. Res., vol. 4, no. 1, pp. 74–79, 2014.
[18] N. K. Narayanan, D. Nargi, C. Randolph, and B. A. Narayanan, "Liposome encapsulation of curcumin and resveratrol in combination reduces prostate cancer incidence in PTEN knockout mice," Int. J. cancer, vol. 125, no. 1, pp. 1–8, 2009.
[19] S. S. Ibrahim, M. A. Barakat, and H. S. Helmy, "Modulating effect of carvedilol on doxorubicin-induced cardiomyopathy and hepatic damage," J Am Sci, vol. 6, no. 12, pp. 20–32, 2010.
[20] S. S. Brar et al., "Reactive oxygen species from NAD (P) H: quinone oxidoreductase constitutively activate NF-κB in malignant melanoma cells," Am. J. Physiol. Physiol., vol. 280, no. 3, pp. C659–C676, 2001.
[21] B. Salehi et al., "Resveratrol: A double-edged sword in health benefits," Biomedicines, vol. 6, no. 3, p. 91, 2018.
[22] C. Colica, M. Milanović, N. Milić, V. Aiello, A. De Lorenzo, and L. Abenavoli, "A systematic review on natural antioxidant properties of resveratrol," Nat. Prod. Commun., vol. 13, no. 9, p. 1934578X1801300923, 2018.
[23] G. Passarino, G. Rose, D. Bellizzi, M. De Luca, and E. S. Gonos, "Aging and longevity between genetic background and lifestyle intervention." Hindawi, 2014.
[24] J. Yu, C. Wang, Q. Kong, X. Wu, J.-J. Lu, and X. Chen, "Recent progress in doxorubicin-induced cardiotoxicity and protective potential of natural products," Phytomedicine, vol. 40, pp. 125–139, 2018.
[25] A. F. Wali et al., "Naringenin regulates doxorubicin-induced liver dysfunction: impact on oxidative stress and inflammation," Plants, vol. 9, no. 4, p. 550, 2020.
[26] Y. I. Kandil, A. D. Maraqa, G. A. Oriquat, and Z. A. Shraideh, "Resveratrol pretreatment reduces circulating inflammatory interleukins in CCl4-induced hepatotoxicity rats," Bull. Fac. Pharmacy, Cairo Univ., vol. 55, no. 2, pp. 319–323, 2017.
[27] D. de Sá Coutinho, M. T. Pacheco, R. L. Frozza, and A. Bernardi, "Anti-inflammatory effects of resveratrol: mechanistic insights," Int. J. Mol. Sci., vol. 19, no. 6, p. 1812, 2018.
[28] Z. Bai and Z. Wang, "Genistein protects against doxorubicin-induced cardiotoxicity through Nrf-2/HO-1 signaling in mice model," Environ. Toxicol., vol. 34, no. 5, pp. 645–651, 2019.
[29] J. A. Araujo, M. Zhang, and F. Yin, "Heme oxygenase-1, oxidation, inflammation, and atherosclerosis," Front. Pharmacol., vol. 3, p. 119, 2012.
[30] E. Gammella, F. Maccarinelli, P. Buratti, S. Recalcati, and G. Cairo, "The role of iron in anthracycline cardiotoxicity," Front. Pharmacol., vol. 5, p. 25, 2014.
[31] T. Farkhondeh, S. L. Folgado, A. M. Pourbagher-Shahri, M. Ashrafizadeh, and S.

Samarghandian, “The therapeutic effect of resveratrol: Focusing on the Nrf2 signaling pathway,” Biomed. Pharmacother., vol. 127, p. 110234, 2020.
[32] Q.-L. Zhang, J.-J. Yang, and H.-S. Zhang, “Carvedilol (CAR) combined with carnosic acid (CAA) attenuates doxorubicin-induced cardiotoxicity by suppressing excessive oxidative stress, inflammation, apoptosis and autophagy,” Biomed. Pharmacother., vol. 109, pp. 71–83, 2019.
[33] Y. Ruan et al., “SIRT1 suppresses doxorubicin-induced cardiotoxicity by regulating the oxidative stress and p38MAPK pathways,” Cell. Physiol. Biochem., vol. 35, no. 3, pp. 1116–1124, 2015.
[34] A. De Angelis et al., “SIRT1 activation rescues doxorubicin-induced loss of functional competence of human cardiac progenitor cells,” Int. J. Cardiol., vol. 189, pp. 30–44, 2015.
[35] C.-J. Chen, W. Yu, Y.-C. Fu, X. Wang, J.-L. Li, and W. Wang, “Resveratrol protects cardiomyocytes from hypoxia-induced apoptosis through the SIRT1–FoxO1 pathway,” Biochem. Biophys. Res. Commun., vol. 378, no. 3, pp. 389–393, 2009.
[36] N. A. T. Abbas and S. L. Kabil, “Liraglutide ameliorates cardiotoxicity induced by doxorubicin in rats through the Akt/GSK-3β signaling pathway,” Naunyn. Schmiedebergs. Arch. Pharmacol., vol. 390, no. 11, pp. 1145–1153, 2017.
[37] L. B. Shi et al., “Transforming growth factor beta1 from endometriomas promotes fibrosis in surrounding ovarian tissues via Smad2/3 signaling,” Biol. Reprod., vol. 97, no. 6, pp. 873–882, 2017.
[38] X. Sun et al., “Qiliqiangxin improves cardiac function and attenuates cardiac remodelling in doxorubicin-induced heart failure rats,” Pharm. Biol., vol. 58, no. 1, pp. 417–426, 2020.
[39] Y. Zhang et al., “Resveratrol inhibits the TGF-β1-induced proliferation of cardiac fibroblasts and collagen secretion by downregulating miR-17 in rat,” Biomed Res. Int., vol. 2018, 2018.
[40] S. A. Serna-Salas et al., “Doxazosin and carvedilol treatment improves hepatic regeneration in a hamster model of cirrhosis,” Biomed Res. Int., vol. 2018, 2018.

Figure Legend

Figure (1). Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES reduced serum ALT in DOXR-intoxicated rats. Data are expressed as mean ± SEM, (n = 6). *** p 0.001

Figure (2) Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES restored hepatic MDA and GSH levels in DOXR-intoxicated rats. Data are expressed as mean ± SEM, (n = 6). *** p 0.001, ** p 0.01, * p 0.05, ns- nonsignificant.

Figure (3) Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES decreased hepatic DNA fragmentation in DOXR-intoxicated rats. Data are expressed as mean ± SEM, (n = 6). *** p 0.001.

Figure (4) Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES downregulated the expression of hepatic TNF-α and IL-6 in DOXR-intoxicated rats. Data are expressed as mean ± SEM, (n = 6). *** p 0.001, ** p 0.01, * p 0.05, ns- nonsignificant.

Figure (5) Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES modulated hepatic HO-1 and ferritin gene expression. Data are expressed as mean ± SEM, (n = 6). *** p 0.001, ** p 0.01, * p 0.05.

Figure (6) Carvedilol (CAR) and/or resveratrol (RES) and LIPO-RES ameliorated hepatic protein expression of SITR and TGF-β1. Data are expressed as mean ± SEM, (n = 6). *** p 0.001, ** p 0.01, * p 0.05.

Figure **(7)** (A) liver section from normal control group showed hepatic tissue with normal architecture, hepatocytes arranged in thin plates (black arrow), with normal sinusoids (yellow arrow) and dilated central vein (red arrow). (B) DOXR group showed preserved lobular hepatic architecture, hepatocytes arranged in thin plates (black arrow), congested sinusoids (yellow arrow), binucleated hepatocytes (green arrow), portal tract vein congestion (white arrow). (C) CAR group showed hepatic tissue with normal structure, hepatocytes arranged in thin plates with mild hydropic changes (black arrow), congested sinusoids (yellow arrow), and dilated central vein (red arrow). (D) RES group showed hepatic tissue with normal structure and architecture, hepatocytes arranged in thin plates with mild hydropic changes (black arrow), dilated sinusoids (yellow arrow), congested central vein (red arrow), binucleated nuclei (green arrow), and aggregates of lymphocytes at portal tract (blue arrow). (E) LIPO-RES group showed hepatic tissue with almost normal structure, hepatocytes arranged in thin plates (black arrow) and dilated central vein (red arrow), binucleated hepatocytes (yellow arrow), and congested portal vein (green arrow). (F) CAR+RES group showed hepatic tissue with normal structure, hepatocytes arranged in thin plates (black arrow) and dilated sinusoids (yellow arrow), dilated central vein (red arrow), and congested portal vein (green arrow). (G) CAR+LIPO-RES group showed hepatic tissue with normal structure, hepatocytes arranged in thin plates (black arrow) and dilated sinusoids (yellow arrow), dilated central vein (red arrow). (H&E x400).