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Protective Effects of Dimethyl-Fumarate Against Doxorubicin-induced Cardiac Injury in Rats

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Abstract

Doxorubicin (DOX) is a broad-spectrum antineoplastic agent; however, doxorubicin's associated cardiotoxic adverse effect through oxidative damage and apoptosis limits its clinical application. Dimethyl Fumarate (DMF) is an FDA-approved treatment for multiple sclerosis shown to have antioxidant, anti-inflammatory and antimutagenic effects via activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. The present study aimed to investigate the potential chemoprotective effect of DMF on Dox-induced cardiotoxicity in rats. Thirty-two Wistar Albino rats of both sexes were administered DMF (15 mg/kg /day) for 14 consecutive days by oral gavage alone or with doxorubicin which was injected as a single dose (15 mg/kg intraperitoneally at day 14) to induce toxicity. The result showed that DMF significantly improved cardiac injury induced by doxorubicin, seen as decreased serum levels of CK-MB and LDH and improved histopathological changes. In addition, DMF significantly inhibited DOX-induced oxidative stress by reducing the level of malondialdehyde (MDA) and increased glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase-1(Gpx-1) cardiac tissue levels; DMF significantly enhanced Nrf2 gene expression and promoted the expression of downstream antioxidant heme-oxygenase-1 (HO-1) gene, downregulated Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor kappa B (NF-κB) genes expression; in addition, DMF significantly reduced inflammatory mediators TNF-α, IL-1β levels and inhibited cardiac apoptosis by modulating bcl-2-like protein (Bax) and caspase-3 tissue levels. In conclusion, the current study established that DMF alleviated the cardiac injury stimulated by DOX via modulating apoptotic responses and reaction to oxidants by stimulating Nrf-2; suggesting that DMF might be used as a possible chemotherapeutic-adjuvant to improve anthracyclines-related cardiotoxicity.

Keywords: Doxorubicin, Dimethyl Fumarate, oxidative stress, Nrf2 pathway, cardiac injury.

Introduction

Doxorubicin (DOX), known as "Adriamycin", is a member of the anthracycline's antibiotics with a broad antitumor spectrum, where it is used worldwide in the treatment of many human malignant cancers as a component of various chemotherapeutic regimens such as breast carcinoma, soft tissue sarcomas, small-cell lung carcinoma, and haematological malignancies [(1)]. DOX antitumor mechanism involves a direct interaction with DNA, thus interfering with DNA replication-related enzymes function mainly topoisomerase-II (Top-II)[(2,3)]; in addition, DOX depletes antioxidants and increases lipid, and protein peroxidation, by overproduction of ROS and oxidative stress which influence both cancerous and normal cells [(4,5)]. Consequently, DOX induces dose-dependent toxicities including severe cardiotoxicity[(6,7)]. The mechanism that contributes to DOX-induced cardiotoxicity is uncertain, but it seems to be separate to some extent from the activity that is related to DOX's anti-tumour activity; nonetheless, cardiac damage is thought to be linked to oxidative damage, autophagy and apoptosis; in which the lipid peroxidation, disordered Ca^{2+} regulation, mitochondrial injury, and modified proinflammatory cytokines production might account for the leading cause of cardiomyopathy [(8-10)]. The Kelch-like ECH-associated protein 1 (Keap1) (Nrf2-inhibitor)/nuclear factor erythroid 2-related factor 2 (Nrf2) / antioxidant response elements (ARE) pathway has been shown to play a part in the body's defence against diseases including cardiovascular diseases including myocardial ischemia, fibrosis and reperfusion myocardiopathy injury, wherein Nrf2 is a key transcriptional element that modulates the antioxidant cellular response by controlling the expression of antioxidant genes[(11)] .

Therefore, studies explored the opportunity to alleviate DOX-mediated cardiac injury by offsetting oxidative stress via activating endogenous antioxidant mechanisms [12,13]. While dexrazoxane presently is the only agent certified to counteract and cope with cardiotoxicity produced by DOX, however, its use is associated with a considerable possibility of inducing haematological abnormalities, which restricts its use with chemotherapy [14,15].

DMF is a fumaric-acid-di-methyl ester-derived agent found in *Fumaria officinalis* leaves known as the "earth smoke" plant; DMF is considered diseases modifying drug used to manage relapsing-multiples sclerosis and psoriasis [16]. Its anti-oxidant, anti-inflammatory and antitumoral activities are thought to involve the activation of the Nrf2, which controls the expression of various genes that regulate antioxidant and detoxification processes [17] by employing a dual effect on Nrf2 activation through not only interrupting Keap1/Nrf2 interaction but also by decreasing Nrf2 degradation [18,19]. Various studies have shown that DMF increased Nrf2 activation and expression, which induced heme oxygenase 1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1), suppressed NF-kB, apoptosis and autophagy [20–23].

Hence, the current work was introduced to investigate dimethyl fumarate's potential cardioprotective role on DOX-mediated cardiac damage in rats at a low dose of 15mg/kg.

Materials and Methods

Drug and chemical

DMF (CAT number-624-49-7) was purchased from Sigma (St. Louis, US). Doxorubicin (Doxorubicin hydrochloride 50mg powder for injection, Khandelwal Labs, India) was purchased from a local pharmacy. Polysorbates (Tween-20) was from Sinopharm Chemicals, China. solvents of the highest grades were utilized.

Formulation and treatment method

DOX powder dissolved in 0.9% NaCl, with an only-once dose of 15mg/kg dispensed as IP injection following previous studies on inducing cardiotoxicity [24].

Furthermore, DMF was freshly suspended using 5% tween-20 in distilled water (D.W.); it was orally administered once daily at 15mg/kg [25].

Investigational protocol

Thirty-two Wister albino rats of both sexes weighing 140-150 g at 6 weeks old were utilized; animals were acquired and maintained in the College of Pharmacy Experimental Animal House, University of Baghdad, Iraq; kept in measured environments of a 12 hours-cycle (light and dark), temp. (22±2 °C), humidity (55±3%). had free access to standard diets and water. Rats were acclimatized up to 1 week preceding the start of the study. The study protocol was approved by the Graduate Studies and Ethics Committees of the College of Pharmacy/ University of Baghdad.

Rats were allocated to 4 groups in this experiment (N= 8, each group) as described below:

Group I: Rats were given (5% tween in D.W.) as a vehicle orally, for 14 days successively This group served as the normal (negative control) group.

Group II: Rats were given orally DMF (15mg/kg/day) for 14 consecutive days.

Group III: Rats were given vehicle-only for 14 successive days. Followed by DOX (15 mg/kg/ single dose) was IP-injected 1 hour after the last vehicle administration on day 14 to serve as the positive control group.

Group IV: Rats given DMF (15mg/kg/day) orally for 2 weeks, followed by DOX (15 mg/kg) was IP-inj. once on the last day of the experiment 1 hour after the last DMF treatment.

Serum collection and tissue processing

After 24 hrs. following DOX administration (day 15), the animals were put under anaesthesia, Jugular vein blood was collected into a test tube, then centrifugated for 15 min at 5000 rpm to obtain blood sera and then kept at -21°C. The rats were sacrificed by cervical dislocation, rats' hearts were removed, washed with cold PBS; and sectioned into several parts, one portion was homogenized in a cold buffer (1: 10), and the supernatant was frozen at −21°C. An additional piece was preserved in 10 %formalin for histological assessment [26,27].

Evaluation of cardiac damage indicators

Creatine kinase myocardial band (CK-MB) and Lactate Dehydrogenase (LDH) levels and other tissue indicators, involving ALT, AST, and ALP levels, were assessed spectrophotometrically using accessible commercial kits (Linear Chemicals, Spain), using HumaReader-HS (Human, Germany).

Estimation of Oxidative-Stress, Inflammation and Apoptotic parameters

Oxidative/antioxidant parameters significance was measured by quantifying malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx-1) levels; also, by mean of a commercial-available ELISA kit, the IL-1β and TNF-α-levels were measured [28,29].

The activity of casp3 and Bax levels was measured by ELISA kits acquired from MyBioSource, US per the manufacturer's instructions.

RT-qPCR analysis

TransZol-Up Plus-RNA Kit was utilized to extract Total-RNA from the cardiac tissue; cDNA was manufactured by EasyScript-One-Step Super-Mix-Kit (TransGen, China), and utilizing Green qPCR SuperMix to quantify mRNA in the presence of Forward and Reverse primer sets of the gene of interest. The comparative alteration in mRNA expressions was equalized to GAPDH-level and calculated by ΔΔCt "Livak" method [30]. Primers

were designed using 'Primer Quest' (IDT, USA); the primer sequence for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Nuclear factor erythroid 2-related factor 2 (Nrf2), Heme Oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase (NQO1), Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor kappa B (NF-κB) are shown in Table 1. Primers were purchased from Alpha DNA, Canada.

Table 1. Primer sequences for RT-qPCR.

Gene tag	Forward(5′ -3′)	Reverse(5′ -3′)
Nrf2	GCTATTTTCCATTCCCGAGTTAC	ATTGCTGTCCATCTCTGTCAG
HO-1	CTTTCAGAAGGGTCAGGTGTC	TGCTTGTTTCGCTCTATCTCC
NQO1	CTGGCCAATTCAGAGTGGCAT	GAGTGGTGACTCCTCCCAGA
Keap1	AATGCTATGACCCAGACACTG	CAGGTAGTCCAAGTGCTTCAG
NF-κB	AAGCAGGAAGATGTGGTGGA	GATAAGGAGTGCTGCCTTGC
GAPDH	TTCCAGGAGCGAGATCCCGCTAAC	CATGAGCCCTTCCACGATGCCAAAG

Histological evaluation

The heart muscle tissue kept in 10 %formalin, was processed and paraffin fixed; then a ~5µm section was obtained, H&E stained and inspected with light-microscope [31]. Each image obtained was examined and a semiquantitative scoring was performed according to necrosis and inflammation according to a scoring system: score 0, normal; score 1, damage up to 25%; score 2, between 25–50%; score 3, between 50–75%; score 4, above 75% [32].

Statistical analysis

Data were presented as Mean±SD, RT-qPCR data were expressed as mean ± standard error of mean (SEM); ANOVA test was utilized to test the significance between groups, followed by Tukey's post-hoc test for multiple-comparison, GraphPad Prism software (version 9.5.0) was used for this purpose. $P < 0.05$ is deemed to be a significant value.

A. Photomicrographs of H&E (X-400) stained tissues the (*Control*) group has normal histology of Longitudinally oriented cardio-myofibers from the left ventricle of rat, with large oval nuclei containing granular chromatin (black arrows), with faint intercalated discs that are also well demonstrated (blue arrows); The typical branched appearance of the cardiac muscle is also well preserved (yellow arrows). The (DOX) group showed myocardial damage within the longitudinal section in a rat's heart, manifested as areas of pallor in the myocardium with loss of cellular details and necrotic myocardium coalesces, and myocardium coalesces (dotted square), with inflammatory cells and macrophages started to appear (Arrowhead), in addition to oedema and bleeding (denoted as Star). (*DMF*) group cardiac tissue myofibers are well preserved and appear with normal architecture. (*DMF+DOX*) group cardiac tissue general architecture of the left ventricle showed reduced signs of myocardial damage with some losses of cellular architecture and fewer necrotic cells (Hollowed arrow).

B. Histopathological semi-quantification, the grading according to necrotic and inflammatory changes in heart tissue; seven fields were examined. Data presented in (mean±SD) fashion, (N =8).

Results

Effects of DMF on cardiac tissue injury markers levels and histopathological alterations

Figure. 1 showed that IP-administered DOX alone (Group III) caused cardiac injury, as seen in the significant ($p < 0.0001$) elevated serum levels of CK-MB, LDH, AST, ALT, and ALP compared to the control group (Group I). However, Group IV showed a significant reduction in the measured parameters' serum levels compared to the DOX-only group, which indicated that DMF has a protective role against DOX-induced cardiac injury.

Additionally, in (figure. 2), Group III presented highly significant pathological scores as seen with the distorted tissue architecture (disintegrated, fragmented myofibres and non-existent striation), necrotising and inflammatory cellular infiltration compared to Group I that had normal architecture (branched and inosculating muscle fibres) with no necrosis or inflammation. Conversely, Group IV preserved cardiac muscle structure (figure. 2A additionally, DMF pre-treatment at (15 mg/kg b.w.) showed significant ($P<0.0001$) lower cardiac semiquantitative injury scores compared to Group III as demonstrated in (figure 2B). On the other hand, DMF alone (Group II) induced non-significant differences in serum CK-MB, AST, ALT, and ALP with a significantly reduced LDH level; In addition, DMF alone causes no histopathological alterations in the cardiac tissue (Figure. 2A), with a score analogous to group I (Figure. 2B).

Effects of DMF on Oxidant/Antioxidant Parameters

The cardiac antioxidant activity of DMF was assessed in rats following acute DOX exposure, as oxidative stress is important in DOX-mediated myocardial damage. Administration of DOX alone (Group III) caused a significant increase ($p<0.0001$) in the MDA tissue level compared to the control group (Group I); However, pre-treatment with DMF

(Group IV) significantly ameliorated (p<0.0001) the increase in MDA cardiac tissue level (Figure. 3A). on the other hand, DOX alone (Group III) caused a significant GSH depletion and SOD, CAT and GPx-1 exhaustion compared to the control group (Group I**),** while pre-treatment with DMF (Group IV) significantly increased the levels of GSH and restored SOD, CAT, and GPx-1 activity in comparison to DOX group (Group III) [Figure. 3B-E].

Furthermore, DMF alone (Group II) caused no significant difference in MDA level (p > 0.05) (Figure. 3A) and significantly increased (p<0.001) cardiac tissue level of SOD compared to the control group (Group I) (Figure. 3C).

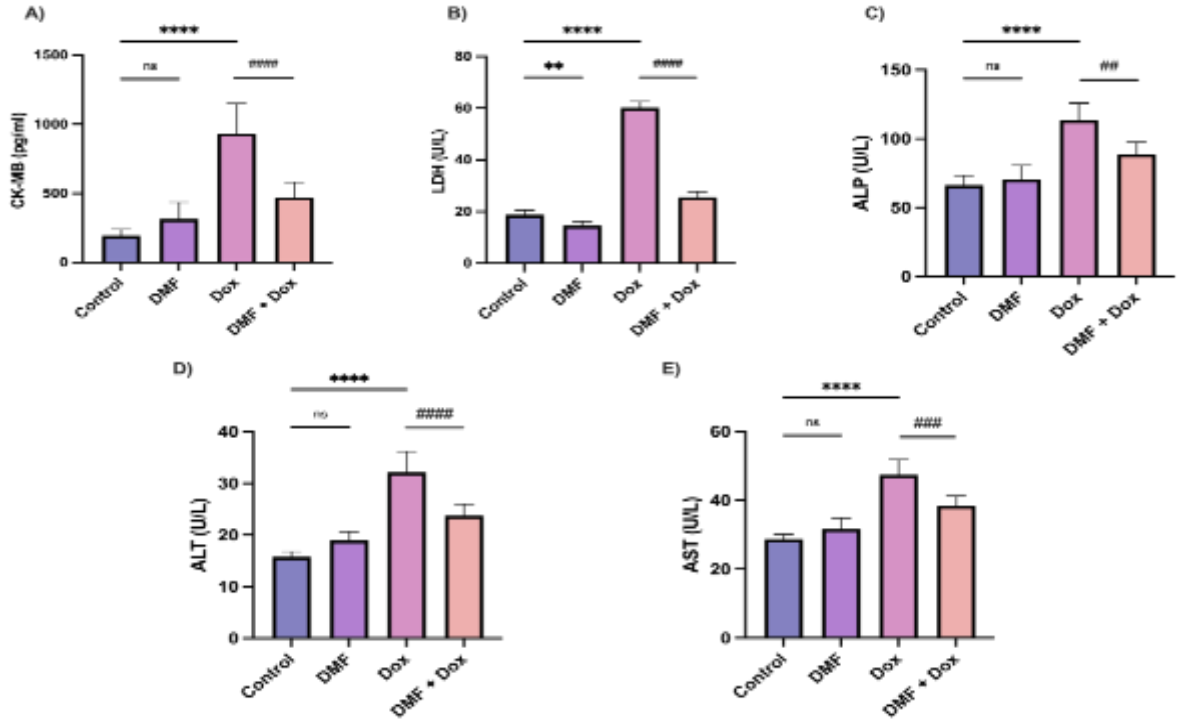

Figure 1. DMF effect on injury markers in rats. A. Effects on CK-MB serum level. B. Effects on LDH serum level. C. Effects on ALP serum level. D. Effects on ALT serum level E. Effect Effects on AST serum level. Data are expressed as (mean±SD), (n=8). ** (P<0.01), **** (P<0.0001) vs. control group; ## (P<0.01), ### (P<0.001), #### (P<0.0001) vs. DOX group. ns (P>0.05), no significant difference.

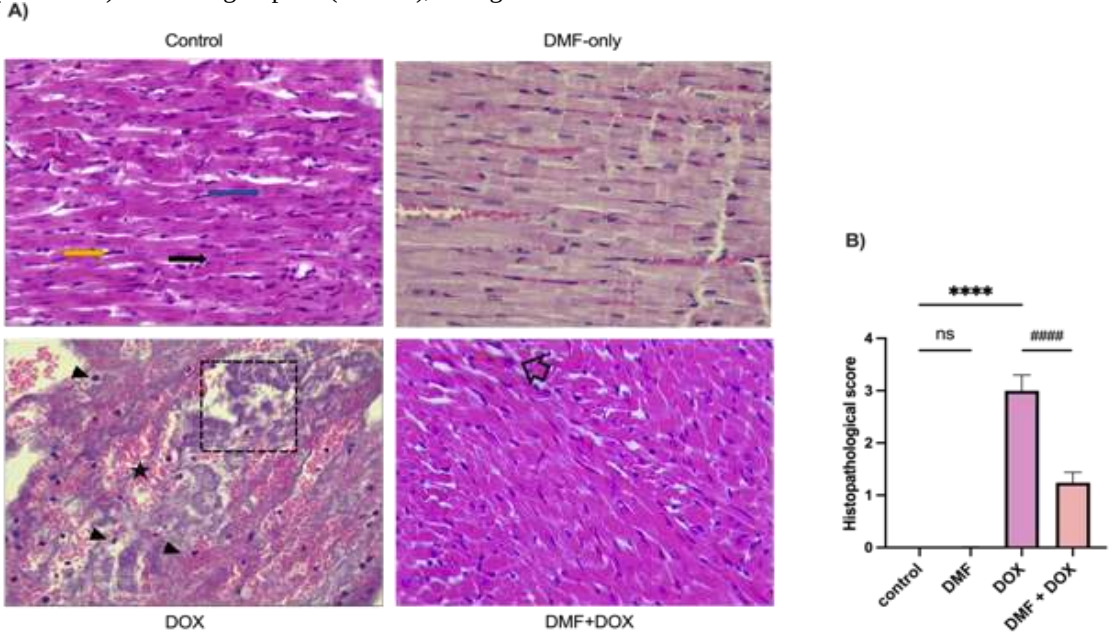

Figure 2. DMF effects on histological alteration in rats' heart.

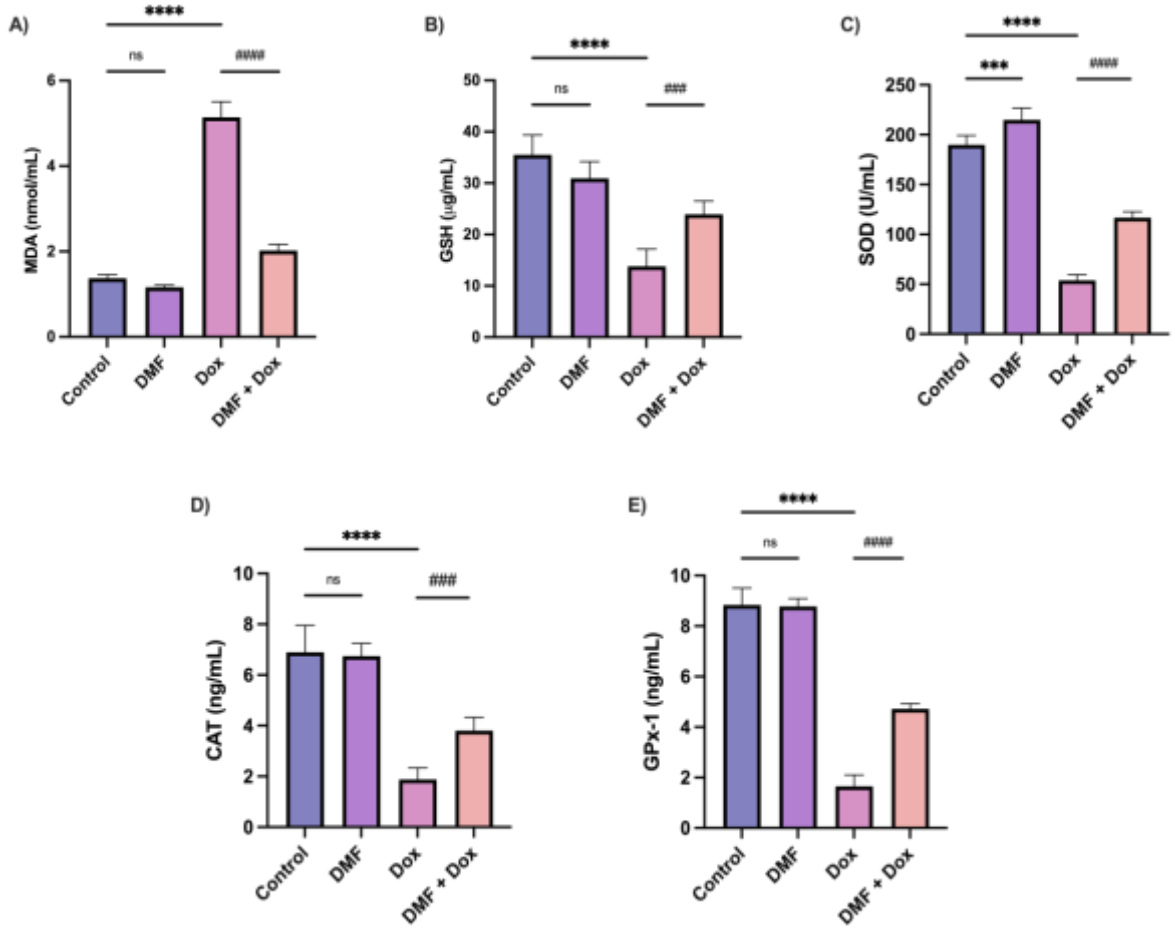

Figure 3. DMF effects on Oxidant/antioxidant parameters in rats' heart . A. Effects on level of MDA. B. Effects on level of GSH. C. Effects on level of SOD.D. Effects on level of CAT. E. Effects on level of GPx-1. Data are expressed as (mean±SD), (n=8). *** (P<0.001),**** (P<0.0001) vs. control group; ### (P<0.001), #### (P<0.0001) vs. DOX group; ns (P>0.05), no significant difference.

Effects of DMF on IL-1β & TNFα tissue levels and NF-κB expression

Proinflammatory cytokines (IL-1β and TNF-α) cardiac tissue levels and NF-κB gene expression were analysed as Inflammation is one of the causes of cardiomyocyte death and subsequent cardiac dysfunction, Figure. 4 A&B, showed that there was a significant increase (P<0.0001) in the cardiac tissue level of TNF-α and IL-1β in the DOX group compared to the control group. Additionally, the expression of the NF-κB gene in the cardiac tissue significantly increased (P<0.01) in the model group (group III), as seen in (Figure. 4 C). However, DMF pre-treatment in Group IV significantly reduced cardiac tissue levels of TNF-α and IL-1β and inhibited the expression of the NF-κB gene compared to the model group (Group III).

DMF alone (Group II) showed comparable cardiac levels of TNF-α , IL-1β and NF-κB gene expression to that of the negative control group (Group I).

Effects of DMF on apoptotic indicators

Apoptotic cell death of the cardiomyocytes is a causative factor of heart failure following the use of doxorubicin; thus, we examined the cardiac tissue levels of both caspase 3 and Bax. As shown in (figure. 5 A&B), there was a significant increase (P<0.0001) in the cardiac tissue levels of both casp 3 and Bax in the model group (Group III) compared to the control group (Group I); while pre-treatment with DMF prior to acute exposure to DOX (Group IV) induced a significant inhibition in the apoptotic markers compared to Group III; while DMF alone (Group II) has no significant effect on Casp3 and Bax cardiac tissue levels compared to the control group.

Effects of DMF on Nrf2 pathway genes expression

Figure 6 showed that the Nrf2 and HO-1 genes expression were significantly decreased (P<0.0001) and (P<0.01), respectively, and there was a significant increase (P<0.01) in the expression of the Keap1 gene in the model group (Group III) compared to Group I. Also, doxorubicin alone

caused a non-significant change in the NQO-1 gene expression in comparison to Group I (control group).

However, the mRNA expression levels of Nrf2, HO-1 and NQO-1 in group IV (DMF pre-treatment + DOX) were significantly higher (P<0.0001) compared to group III (doxorubicin only); in addition, DMF pre-treatment significantly reduced (P<0.0001) the cardiac tissue gene expression of Keap1 compared to Group III. On the other hand, DMF alone (Group II) significantly increased cardiac tissue mRNA levels of HO-1 and NQO-1 with no significant effect on Nrf2 and Keap1 levels compared to the control group.

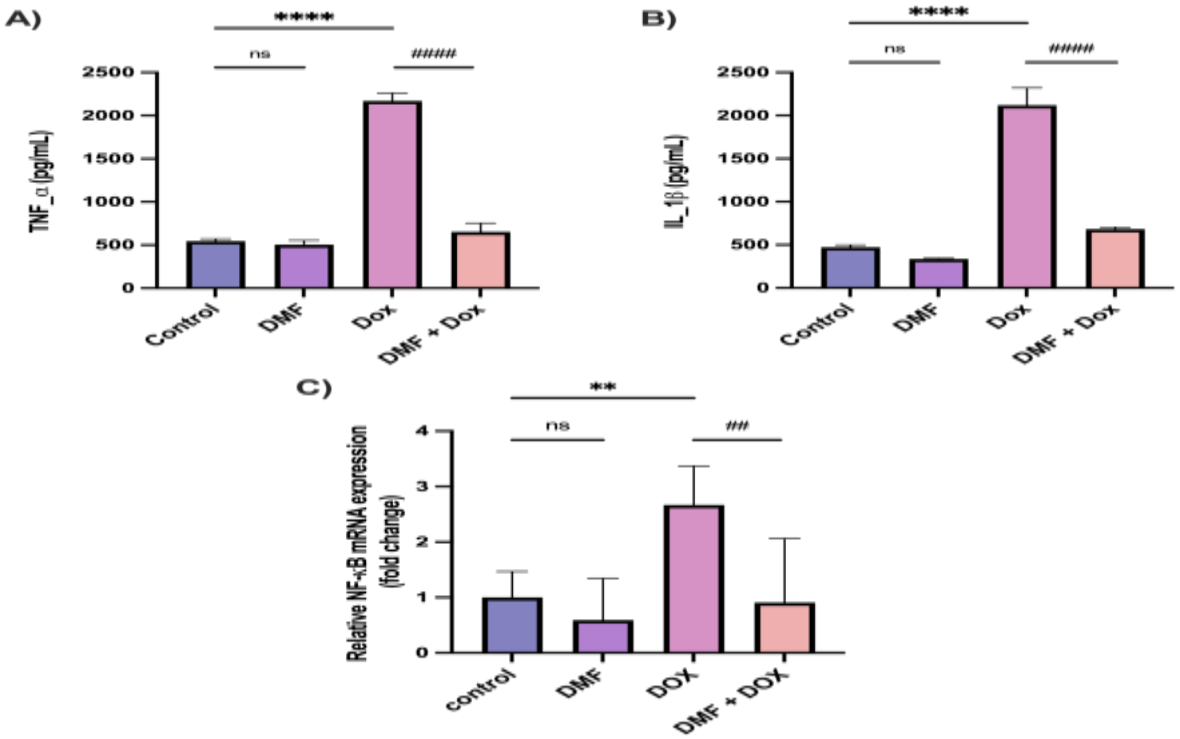

Figure 4. DMF effect on proinflammatory indicators in rats' heart. A. Effects on TNF-alpha. B. Effects on IL-1beta. (means±SD) used to represent data, (n=8).C. Effects on NF-κB. Data are expressed as (mean±SEM), (n=8). ** (P<0.01), **** (P<0.0001) vs. control group; ## (P<0.01), #### (P<0.0001) vs. DOX group; ns (P>0.05), no significant difference.

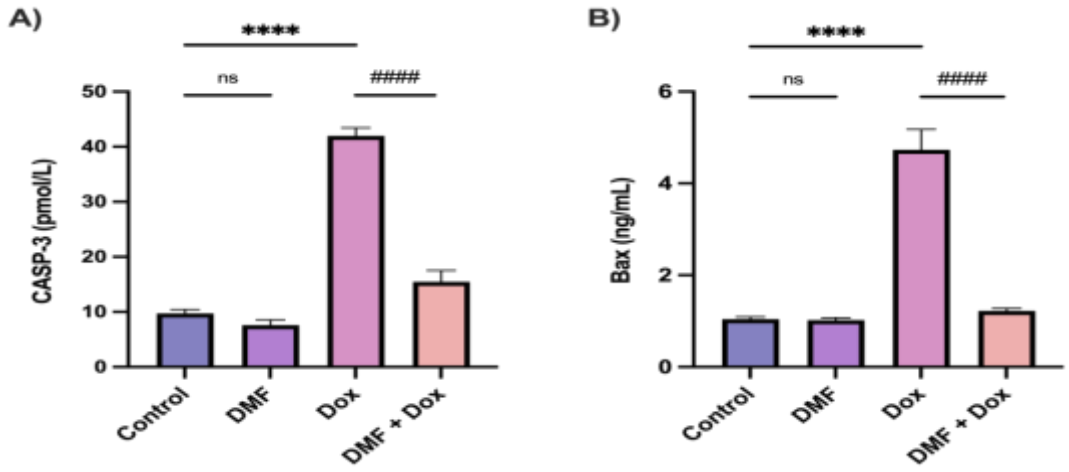

Figure 5. DMF effects on apoptotic indicators in rats' heart. A) Effect of DMF on Casp3 cardiac tissue level. B) Effect of DMF on Bax cardiac tissue level.Data are expressed as (mean±SD), (n=8).**** (P<0.0001) vs. control group; #### (P<0.0001) vs. DOX group; ns (P>0.05), no significant difference.

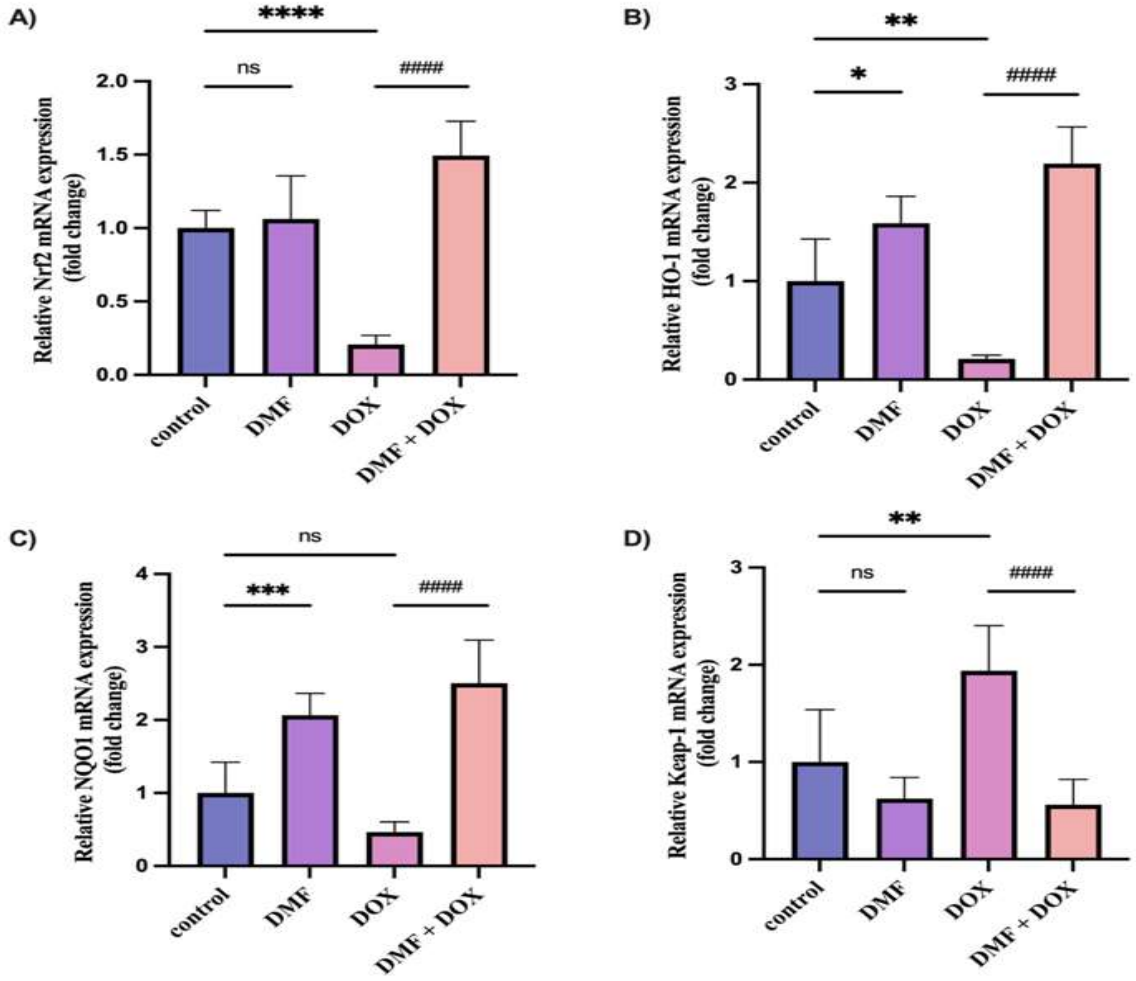

Figure 6. DMF effects of Nrf2 pathway elements in rats heart.A) Effect of DMF on Nrf2 gene expression. B) Effect of DMF on HO-1 gene expression. C) Effect of DMF on NQO1 gene expression. D) Effect of DMF on Keap1 gene expression.Data are expressed as (mean±SEM), (n=8).* (P<0.05), ** (P<0.01), ***(P<0.001), **** (P<0.0001) vs. control group; #### (P<0.0001) vs. DOX group; ns (P>0.05), no significant difference.

Discussion

Although DOX is used for treating a broad range of solid tumours, its use is associated with severe cardiotoxicity, including arrhythmia, reduced ejection fraction, cardiomyopathies and heart failure [33]. The mechanism that mediates doxorubicin-induced cardiotoxicity is unclear; however, it might be related to oxidative stress, inflammatory cascade, apoptosis and DNA damage; among them, oxidative stress plays an important role in DOX-induced toxicities through the generation of free radicals that cause the depletion of the antioxidants, increasing lipid, protein and nucleic acid peroxidation, and disturbing mitochondrial function [34 ,4, 7,9]. As Nrf2 plays a critical role in regulating oxidative stress within cells, once oxidative stress occurs, Nrf2 translocate into the nucleus after dissociating from Keap1 due to the oxidation of the active site of Keap1, triggering the expression of different antioxidant genes; in addition, Nrf2 signalling pathway activation can efficiently preserve cellular redox homeostasis , modulate apoptotic proteins level, with an efficient anti-inflammatory function which helps alleviate myocardial infarction and other cardiovascular disorders [35,36].

In the current study, the data showed that acute exposure to DOX produced cardiac injury, represented by the change in the histological appearance of cardiac tissue and the significant increase in serum levels of CK-MB, LDH and other tissue injury markers as indicators of clinical myocardial injury; in addition, DOX caused oxidative stress as seen in the elevated cardiac tissue level of MDA, depleted GSH and antioxidant enzymes; induced the apoptosis and inflammatory response which was observed by increasing the Bax and caspase-3, and TNF-α and IL-1β cardiac tissue levels. Furthermore, DOX alone suppressed the Nrf2 pathway by enhancing Keap1 gene expression and reducing Nrf2 and HO-1 gene tissue levels compared to Group I (negative control group). These results are agreeable with previous studies affirming that DOX caused cardiac damage, oxidative stress, and induced apoptosis and

inflammation [12,24,37,38] thus, DOX-induced cardiotoxicity was successfully established [31].

The results revealed that dimethyl fumarate (15mg/kg/day) alone caused no significant difference in CK and LDH serum levels with a similar cardiac tissue histological appearance to that of the control group; this indicates that DMF alone has no cardiotoxic effect on rats' heart *in vivo*.

While DMF pretreatment prior to DOX acute exposure efficiently protected against DOX-induced cardiac injury as it significantly reduced the serum levels of CK-MB and LDH, ameliorated the histopathological changes and significantly reduced the apoptosis and inflammatory markers; Furthermore, DMF pre-treatment significantly increased the cardiac tissue levels of GSH, SOD, CAT, and GPx-1, reduced MDA tissue level and promoted Nrf2, and HO-1 genes expression and significantly decreased the expression of the Keap1 gene expression thus successively activated Nrf2 pathway and inhibited the oxidative stress-mediated damage in cardiomyocytes when compared to animals treated with doxorubicin alone; therefore, DMF might have a beneficial role in alleviating doxorubicin-mediated acute cardiac injury. Our results are in agreement with the previously reported cardioprotective effect of DMF against doxorubicin-induced cardiotoxicity through the activation of the Nrf2 pathway [26].

The mechanism that may explain the reduction of doxorubicin-induced cardiotoxicity might be related to the DMF protective effect as a result of its antioxidant activity through the activation of the Nrf2 pathway [8][20]. Studies have explained in CVD including ischemic heart damage (IHD) and the subsequent cardiac dysfunction, that the most critical feature is the overproduction of ROSs, which results in lipid peroxidation and cardiomyocyte oxidative damage, and eventually-leading to apoptosis; in addition, researchers reported that the Nrf2 overexpression achieved a protective effect against myocardial ischemia; while the size of myocardial infarction (MI) area was reported to increase in Nrf2 knockout mice following IR reducing its cardioprotective activity [39]. As an Nrf2 activator, DMF has previously been shown to alleviate cardiac oxidative stress damage by inhibiting doxorubicin-mediated free radicals' generation and apoptosis attenuation [26]; DMF has been used for the treatment of relapsing-remitting multiple sclerosis [40] and is presently considered as an antioxidant that can regulate cellular ROS production and apoptosis [41], DMF showed to have a protective role against myocardial Ischemic/Reperfusion which is related to DMF mediated improved cellular viability, reduced oxidative stress and enhanced the expression of Nrf2-regulated antioxidative genes [42]; in addition, DMF was reported to prevent apoptosis, increase the survival rate and proliferation of human adipose-derived mesenchymal stem cells (hASCs) against oxidative stress which is mediated by upregulation of HO-1 and NQO-1 expression [43].

Conclusion

The data suggested that DMF orally given alone (at a low dose, 15 mg/kg/ day) is safe; DMF treatment before DOX exposure ameliorated cardiac muscle injury, repressed inflammatory response, apoptosis, and oxidative stress by Nrf2-pathway activation; thus, DMF may be a possible used as a chemoprotective agent in chemotherapy.

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Conflicts of Interest

The authors report no conflicts of interest.

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Ethics Statements

The study protocol was approved by the Graduate Studies and Ethics Committees of the College of Pharmacy/ University of Baghdad.

Author Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Sara A., Nada N.; data collection: Sara A.; analysis and interpretation of results: Sara A.; draft manuscript preparation: Sara A., Nada N. All authors reviewed the results and approved the final version of the manuscript.

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التأثير الوقائي للدايمثيل فيوماريت على الضرر القلبي الناجم عن استخدام الدوكسوروبيسين في الجرذان

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الخلاصة

ان الدوكسوروبيسين علاج ذو طيف واسع فعال لعلاج للأورام السرطانية. لكن استخدامه يسبب تأثيرات سامة على القلب من خلال الضرر التأكسدي ، وهذا يحد من تطبيقه السريري. آن الدايمثيل فيوماريت هو علاج معتمد منFDA لمعالجة التصلب المتعدد، وله تأثيرات جديرة بالملاحظة حيث يعمل كمضاد للأكسدة والالتهابات وللطفرات من خلال تنشيط مسار Nrf2. تهدف الدراسة الحالية إلى التحقق من التأثير الوقائي المحتمل لل DMF ضد السمية القلبية المستحثة بالدوكسوروبسين في الجرذان. تم معالجه جرذان من كلا الجنسين ب DMF ١٥ ملغم/كجم يوميًا لمدة ١٤ يومًا متتاليًا عن طريق الفم وحده أو مع دوكسوروبيسين الذي تم حقنه كجرعة وحيدة (١٥ مجم / كجم داخل الصفاق في اليوم ١٤) للحث على السمية.أظهرت النتائج ان استخدام DMF حسن المؤشرات الدالة على الضرر لعضلة القلب CK-MB وLDH المقاسة في مصل الدم نتيجة استخدام دوكسوروبيسين بالإضافة للتحسن الملحوظ في مؤشرات التحليل النسيجي، مع انخفاض ملحوظ بمؤشرات الإجهاد التأكسدي القلبي المتضمنة بانخفاض MDA و زيادة GSHو SOD و CAT وGpx-1 في النسيج القلبي، كما و ان استخدام DMF عزز التعبير الجيني والبروتيني للNrf2 وHO-1 ، و قلل مستويات التعبير الجيني لكل من Keap1وNF-kB و ثبط الموت المبرمج للخلايا القلبية والمتمثل بانخفاض Bax و casp-3، كما وان DMF قلل الالتهاب من خلال تقليل مستويات TNF-a و IL-1b في النسيج القلبي.الدراسة الحالية تستنتج ان استخدام DMF له تأثير وقائي على القلب ضد السمية المستحثة بالدوكسوروبسين من خلال تنظيم الموت المبرمج للخلايا واستجابة الإجهاد التأكسدي عن طريق تفعيل مسارNrf2 ؛ تشير هذه الدراسة إلى أن DMF قد يعمل كمساعد محتمل في العلاج الكيميائي.

الكلمات المفتاحية: دوكسوروبيسين، الدايمثيل فيوماريت، الإجهاد التأكسدي، مسار Nrf2، الضرر القلبي.