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Marjoram (*Origanum majorana* L.) Alleviate Myocardial Damage Induced by Doxorubicin in Rats

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Abstract

The present work evaluated the curative effect of marjoram (*Origanum majorana* L.) (MO) on myocardial toxicity induced by doxorubicin (DOXO) in rats. Forty rats (210-230 g) were allocated into four equal groups; control (CO), cardiotoxic-DOXO (DOXO), marjoram extract (MOE) (750 mg/kg), and marjoram extract (MOE) intoxicated with DOXO (MOE+DOXO). Cardiotoxicity was induced through a single intraperitoneal (i.p.) injection of DOXO 20 mg/kg. Rats received orally MOE (750 mg/kg) for 24 days and at the 21st day, they were injected i.p. with DOXO. Three days (72 h) after DOXO injection, rats in all groups were sacrificed. Ingestion of MOE (750 mg/kg) did not affect myocardial functions; however, there was a slightly non-significant increase in the antioxidant status relative to the CO group. Administration of DOXO significantly induced myocardial damage evidenced through significant increase in serum myocardial functions (CK-MB, and LDH), significant myocardial oxidative stress (significantly increased cardiac TBARS level and significantly decreased cardiac SOD and CAT levels), and inflammation (a significant increase in the serum IL-6 and TNF-α levels) compared to the CO group. Inflammatory lesions, congestion of myocardial blood vessels, focal necrosis of cardiac myocytes, and intramuscular edema were observed in the microscopically examined cardiac tissue. The group pre-treated with MOE (750 mg/kg) before DOXO injection revealed the curative role of MOE as evidenced through a significant improvement in the serum cardiac function, cardiac oxidative stress, and pro-inflammatory cytokines markers relative to DOXO group, as well as showed markedly normalized cardiac tissue. In conclusion, MOE (750 mg/kg) has a curative and cardioprotective role against DOXO through its' potent antioxidant and anti-inflammatory mechanisms. Therefore, this study gives a new curative strategy for cancer patients receiving chemotherapy drugs.

Key words: Myocardial damage, Marjoram extract, Rats, Oxidative stress, Inflammation

Introduction

The anthracyclines and associated compounds (daunorubicin, doxorubicin, epirubicin, idarubicin, and the anthraquinone mitoxantrone) are among the chemotherapeutic agents implicated in cardiotoxicity (Chatterjee *et al*., 2010; Kaya *et al*., 2013). Anthracycline therapy related to an increase in the risk of developing cardiomyopathy, heart failure with significant accompanying mortality and morbidity (Khouri *et al*., 2012).

Doxorubicin (DOXO) is one of the anthracycline cytostatic agents (Hosseini *et al*., 2017). It is an excellent antitumor medication for the treatment of different types of leukemia, solid cancer, and lymphomas (Alkhatib *et al*., 2017). Arrhythmias is one of the symptoms of acute cardiotoxicity, while chronic toxicity progresses to permanent cardiomyopathy, which affects around 30% to 40% of patients with a maximum dose of 5000 mg/mm^2 (Yagmurca *et al.,* 2003). The exact mechanisms underlying the cardiotoxicity of DOXO have not been fully understood.

The effect of DOXO on cardiotoxicity could be explained by different mechanisms. Aryal *et al*. (2014) reported that oxidative stress is partly responsible for cardiotoxicity due to the lack of antioxidant mechanisms in the heart. Other possible mechanisms include apoptosis induction, abnormal extracellular deposition of the matrix, and mitochondrial iron overload (Ichikawa *et al*., 2014). In patients with tumors, the development of novel interventions that can inhibit these pathological changes will reduce or prevent these DOXO-related complications (Kaya *et al*., 2013). Herbal medicines are the most important field of alternative medicines worldwide (Gajalashmi *et al*., 2012; Deepti et al., 2019). In terms of protection, herbal medicines have no side effects and are less expensive than conventional medicines (Andrade *et al*., 2013).

Marjoram (*Origanum Majorana L*.) belongs to the Lamiaceae family (Baranauskienė *et al*., 2005). It is one of the most common kitchen herbs (Srinivasan, 2005). Marjoram's therapeutic properties are tonic, gastrointestinal, diaphoretic, carminative, antibacterial, diuretic, and hypoglycemic (Leeja and Thoppil, 2007) and as an antioxidant (Handl *et al.,* 2008). The antioxidant property of MO as one of the medicinal plants can be linked to its content of polyphenolic compounds including flavonoids (Massoud *et al*., 2009). Gamma-terpinene, terpinen-4-ol, linalool, trans-sabinene hydrate, thymol thujanol, and terpinolene are the essential components of marjoram (El-Ghorab *et al*., 2004). The objective of this study was to investigate the cardioprotective effect of MOE on DOXO-induced cardiac injury in rats.

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Material and Methods

Chemicals

All chemicals with analytical grade used were obtained from Invitrogen Com. and Alfa Aesar Chem. Co. USA

Induction of myocardial toxicity

Doxorubicin® (DOXO) (Adriablastina, 10 mg DOXO hydrochloride/ 5 ml) purchased from Pharacia, Carlo Erba (Sigma Aldrich Inc., USA). Cardiotoxicity was induced through i.p. injection of DOXO 20 mg/kg once, then 72 h after last DOXO injection all rats were sacrificed.

Marjoram hydroalcoholic extraction

Dried Marjoram plant (*Origanum majorana* L.) (MO) purchased from the local marker was grounded. The MO powder (500 g) was macerated in 2 L methanol 70% for 48 h and then extracted 2 times until complete exhaustion. The collected methanolic extract after filtration through a filter funnel was evaporated using a rotary evaporator under reduced pressure at <40 °C and then concentrated by drying the excess water using a freeze dryer at -20 °C for 48 h, and stored at -4°C for further use (Afifi *et al*., 2014). The extract was freshly dissolved in sterile distilled (dis) water with 2 drops of Tween-80 to prepare a final concentration of 1 g/ml.

Experimental protocol

Adult male Albino rats (210-230 g, n=40) were purchased from king Fahd Medical Center, Animal Unit, KAU. After one week of acclimatization under standard lab conditions according to Canadian ethics, they were randomly allocated into four equal groups. Control (CO) group; rats were given orally dis. water and administrated a single i.p. with saline. Cardiotoxic-DOXO (DOXO) group; rats were given orally dis. water and administrated a single i.p. with DOXO on the 21st day. Marjoram extract (MOE); rats were given orally MOE (750 mg/kg) (Afifi *et al.*, 2014) for 24 days. Marjoram extract (MOE) intoxicated DOXO (MOE+DOXO) group; rats were given orally MOE (750 mg/kg) for 24 days and on the 21st day administrated i.p. with a single dose of DOXO. Three days (72 h) after DOXO injection rats in all groups were sacrificed. Serum and heart samples were collected and stored at -80°C until analysis. Heart tissues were either frozen for biochemical analysis or processed for examination for the detection of histopathological alterations.

Estimation of myocardial function

The creatine kinase (CK-MB) and lactate dehydrogenase (LDH) activities were assessed in serum using enzymatic colorimetric kits from Centronic Chem Com, Germany.

Estimation of myocardial tissue oxidative status markers

Thiobarbituric acid reactive substances (TBARS), catalase (CAT), and superoxide dismutase (SOD) were assessed in cardiac tissue using double-antibody Sandwich enzyme-immunosorbent assay ELISA kits (Bioassay Tech Lab, California, USA) according to the kits' manufacturer instructions.

Estimation of myocardial tissue pro-inflammatory cytokines markers

The levels of tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) were assessed in serum using double-antibody Sandwich enzyme-immunosorbent assay ELISA kits (Bioassay Tech Lab, California, USA) according to the kits' manufacturer instructions.

Cardiac histopathological examination

Cardiac tissue was processed and stained with hematoxylin & eosin, then examined under a microscope to detect tissue changes.

Statistical

All results were analyzed using SPSS ver., 24, and represented as mean±standard error (SE). One-way ANOVA, followed by LSD test. The difference was considered significant if $p < 0.05$.

Results

Myocardial function markers

Administration of DOXO significantly altered myocardial functions evidenced through a significant increase in serum CK-MB and LDH levels compared to the CO group. Ingestion of MOE (750 mg/kg) did not affect the myocardial functions compared to the CO group, thus indicated the safety effect of MOE. Regarding myocardial functions in MOE (750 mg/kg)+ DOXO group, there was a significant decline in serum CK-MB and LDH levels relative to the DOXO group.

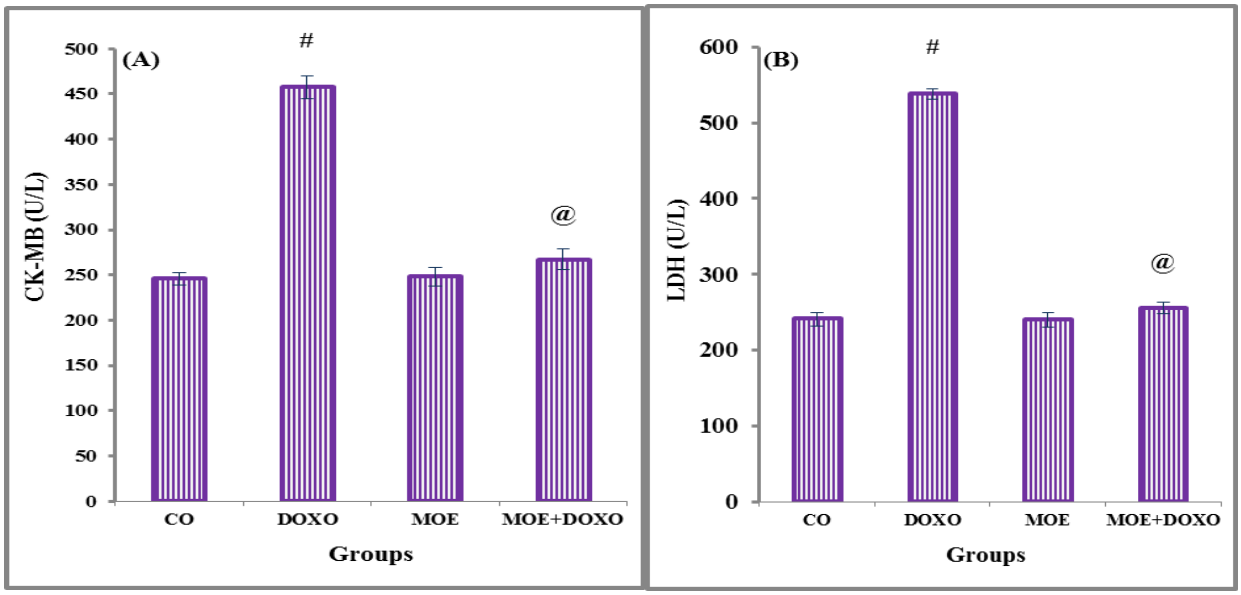

Figure 1: Effect of MOE on the myocardial functions (CK-MB (Fig.A) and LDH (Fig. B) in DOXO-induced cardiac toxicity in rats measured in Control (CO), Cardio toxic Doxorubicin (DOXO), Marjoram extract (MOE) (750 mg/kg), and Marjoram extract (MOE) (750 mg/kg)+ Doxorubicin (MOE + DOXO). Results represented as the mean±SE for 10 rats in each group, [#] significant compared to CO and [@] significant compared to the DOXO group at $p< 0.05$.

Cardiac oxidative status markers

Administration of DOXO significantly induced myocardial oxidative stress evidenced through a significant increase in cardiac TBARS level and a significant decrease in cardiac SOD and CAT concentrations compared to the CO group. Ingestion of MOE (750 mg/kg) induced a slight improvement in the myocardial antioxidant status compared to the CO group, thus indicated the antioxidant action of MOE. There is a significant decline in cardiac TBARS with a significant increase in cardiac SOD and CAT concentrations in the MOE (750 mg/kg)+ DOXO group compared to the DOXO group.

Serum proinflammatory cytokines markers

Administration of DOXO significantly induced inflammation in DOXO-injected rats that was evidenced through significant increases in serum IL-6 and TNF-α levels compared to the CO group. Ingestion of MOE (750 mg/kg) alone induced a slightly non-significant decline in serum levels of IL-6 and TNF-α levels compared to the CO group. In the group pre-treated with MOE (750 mg/kg) before DOXO injection, there was a significant decline in serum IL-6 and TNF-α levels compared to the DOXO group, thus evidenced for the anti-inflammatory role of MOE.

Table 1: The effect of MOE on myocardial antioxidant enzymatic and non-enzymatic contents (SOD, TBARS, and CAT) in DOXO-induced cardiac toxicity in rats measured in Control (CO), Cardio toxic Doxorubicin (DOXO), Marjoram extract (MOE) (750 mg/kg), and Marjoram extract (MOE) (750 mg/kg)+ Doxorubicin (MOE + DOXO) groups.

Groups	SOD (U/mg protein)	TBARS (nmol/mg protein)	CAT (U/mg protein)
CO	4.58 ± 0.43	20.14 ± 1.56	15.93 ± 1.38
DOXO	2.39 ± 0.21[a]	52.94 ± 3.22[a]	8.38 ± 0.73[a]
MOE	4.85 ± 0.39	19.81 ± 1.63	15.54 ± 1.29
MOE+DOXO	4.45 ± 0.26[b]	26.09 ± 1.53[b]	13.02 ± 1.03[b]

Results represented as the mean±SE for 10 rats in each group, [a] significant compared to CO and [b] significant compared to DOXO group at $p< 0.05$.

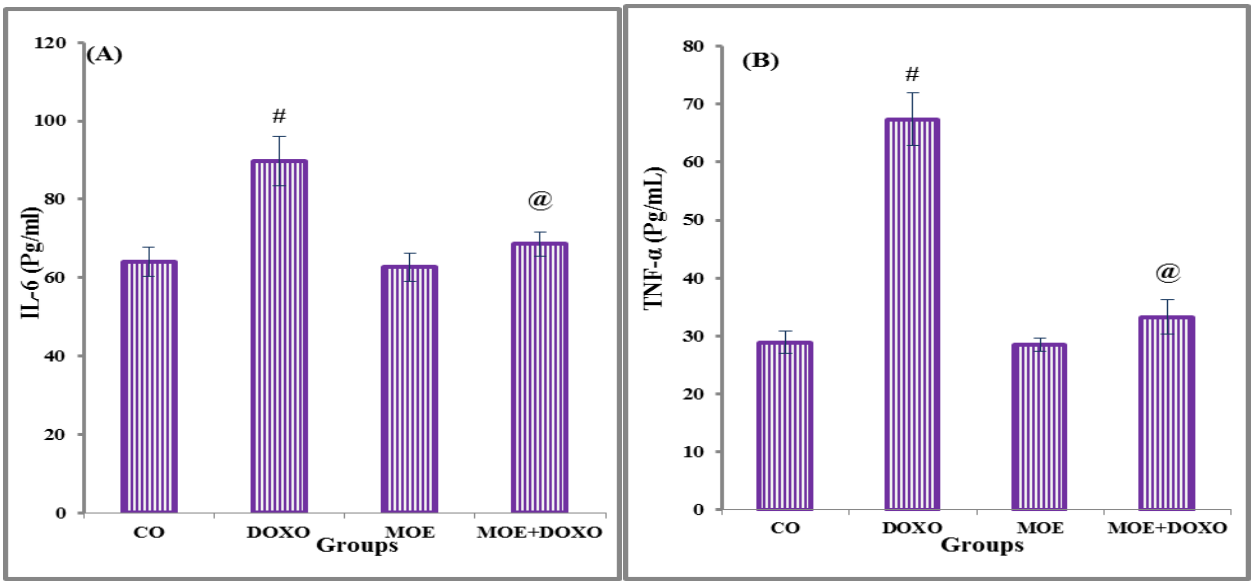

Figure 2: The effect of MOE in serum proinflammatory cytokines (IL-6 (Fig.A) and TNF-α (Fig. B) in DOXO-induced cardiac toxicity in rats measured in Control (CO), Cardio toxic Doxorubicin (DOXO), Marjoram extract (MOE) (750 mg/kg), and Marjoram extract (MOE) (750 mg/kg)+ Doxorubicin (MOE + DOXO) groups. Results represented as mean± SE for 10 rats in each group; # significant compared to CO and @ significant compared to DOXO group at $p< 0.05$.

Myocardial histopathological results

There were normal myocardial myocytes in CO and MOE (750 mg/kg) sections (Fig. A and Fig E, respectively). Administration of DOXO to rats induced inflammatory lesions, congestion of myocardial blood vessels, focal necrosis of cardiac myocytes, and intermuscular edema (Fig. B-D). While in MOE + DOXO group the cardiac myocytes were normal except for slight congestion (Fig. F).

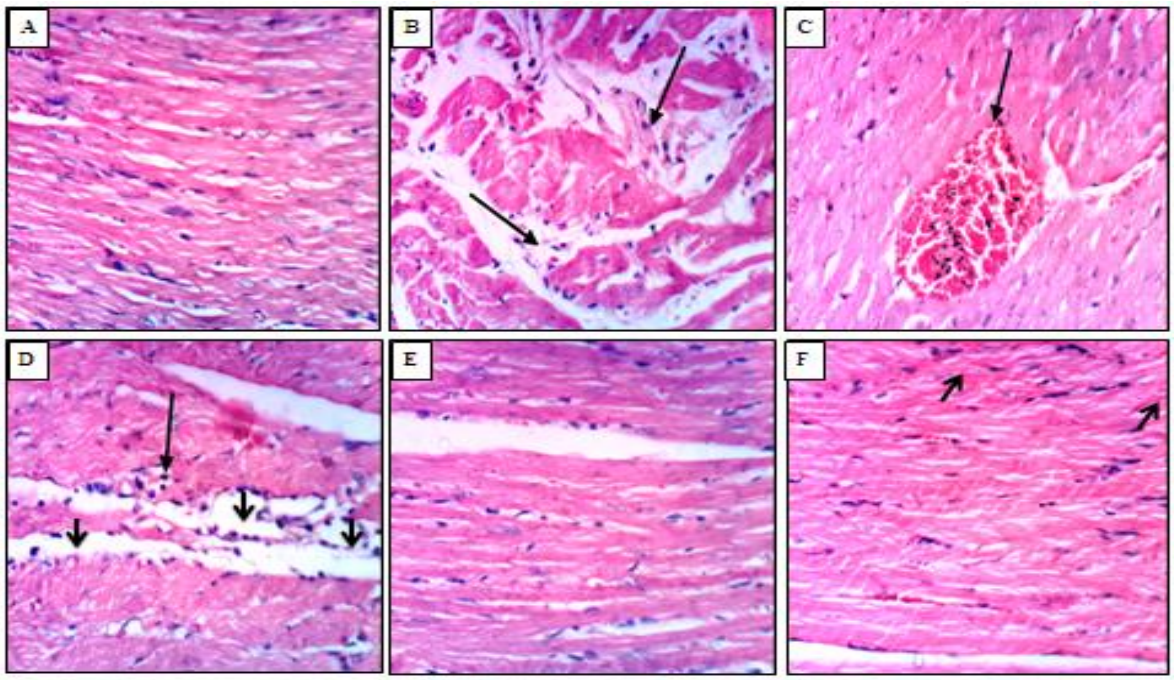

Figure 3: Representative myocardium sections H&E-stained x400. Normal cardiac myocytes in CO (Fig. A), and MOE (750 mg/kg) (Fig. E) groups. In the DOXO group, there are intermuscular edema (Fig. B), inflammatory lesions, and congestion of myocardial blood vessels (Fig. C), focal necrosis of cardiac myocytes with inflammatory cells infiltration (Fig.D). In MOE + DOXO group, cardiac myocytes are normal except for slight congestion (Fig. F).

Discussion

Clinical use of DOXO is conflicted with unwanted side effects, including cardiomyopathy and heart failure congestive (Takemura and Fujiwara, 2007). In many studies, a significant correlation was observed between oxidative stress from DOXO and cardiotoxicity. (Shad *et al*., 2007, Riad *et al*., 2009; Li *et al*., 2009). There are several chronic pathogenic effects of DOXO including iron metabolism, oxidative stress, changes in sarcoma composition, Ca^{2+} homeostasis deregulation, gene expression modulation and apoptosis (Zhu *et al*., 2011). The objective of the current research was to investigate the potential defending effect of MOE on cardiotoxicity in rats induced by DOXO.

The greatest critical mechanism of cardiac toxicity recognized by DOXO is the imbalance between antioxidant enzymes and free radicals (Asensio-López *et al*., 2017). The current results showed significant elevations in LDH and CK-MB, TBARS, IL-6, and TNF-α along with significant decreases in the concentrations of cardiac SOD and CAT following DOXO injection compared to the CO group. Increased serum activity of LDH and CK-MB suggested that DOXO mediated oxidative stress contributing to heart lipid peroxidation; these results are constant with Andreadou *et al*. (2007) and Shah *et al*. (2012).

In addition, DOXO caused oxidative damage to the heart tissue resulting in lipid peroxidation, which evidenced by MDA production along with antioxidant enzyme depletion (SOD and CAT) that created by toxic free radicals (Zhang *et al*., 2005; El-Shitany *et al*., 2008; Shehata 2018). The DOXO's undesirable toxicity is largely due to its intracellular ROS stimulation, which destroys the cell membrane and contributes to dramatic apoptosis (Rice *et al*., 1996; Lu and Foo, 2001). The ROS is also likely to contribute to systemic inflammation during DOXO therapy; however, studies have shown that ROS can significantly increase the level of inflammatory mediators such as IL-6, TNF-α, GM-CSF, and IFN-π. These modifications are related to the harmful effects of reactive oxygen species due to the interaction of DOXO with iron, DNA topoisomerase II inhibition, and activation of certain immune and inflammatory reactions in cardiac tissues (Hosseini *et al*., 2017; Al-Ogaidi 2018). The DOXO interacted with sarcoplasmic reticulum calcium sequestration by altering the cardiomyocyte calcium pump (Childs *et al*., 2002). It also affects the sarcolemma sodium/potassium pump, interfering with the calcium, sodium gradient required to flow into the cardiomyocyte sarcolemma (Fernandez-Chas *et al*., 2018).

The DOXO may interfere with mitochondrial replication and transcription, resulting in major imbalances in the electron transport chain and promoting mitochondrial dysfunction (Floyd *et al*., 2005). In fact, an additional mechanism of cardiotoxicity is given by changing the expression of endothelin-1 in cardiomyocytes after DOXO treatment (Ganz et al., 1996). This may potentiate the increased calcium load of the cardiomyocyte, contributing to cardiac dysfunction (Fernandez-Chas *et al*., 2018).

Herbal medicines have several compounds that use different mechanisms to exert their useful effects on the protection of different diseases (Al-Kuraishy *et al*., 2015). Several medicinal plants have successfully prevented DOXO-related cardiotoxicity (Hosseini *et al*., 2017). The findings of the recent study showed that the oral administration of MOE secured DOXO-intoxicated rats from cardiotoxicity by decreasing the levels of CK-MB, LDH, TBARS, IL-6, and TNF-α along with significant increases in cardiac SOD and CAT concentrations compared to the CO group. In addition, the cardiac histopathology study revealed that MOE prevented DOXO-induced histopathological and ultrastructural damage. The results of the study were in line with Ahmed *et al*. (2009) who reported that the MOE extract significantly reduced the heart tissue content of TBARS and MDA.

Ethyl acetate extract, water extract, and essential oil from the aerial part of MO show substantial antioxidant activity (Mossa and Nawwar, 2011). Other sweet marjoram extracts, including ethanol, n-hexane, and hydroalcoholic extracts have also been reported to have antioxidant properties (Vagi *et al*., 2005). Flavonoids and phenolic compounds such as hydroxycinnamic acid, carnosic acid, ursolic acid, rosmarinic acid, caffeic acid, and carnosol are responsible for antioxidant activity (Hossain *et al*., 2014). El-Moursi *et al*., (2012) confirmed that the MOE has several antioxidant compounds such as highly labile carnosol, carnosic acid, followed by flavonoids and apigenin-7-O-glucoside. In addition, MOE contains a high amount of ursolic acid (Erenler *et al*., 2016; Hasan, 2019.). Terpineol and sabinene in MO s suppress the tumor necrosis production as IL-1β, IL-10, IL-6, and TNFα which inhibit the expression of NFπB genes, and cyclooxygenase 2 (COX2) (Arranz *et al*., 2015).

In addition, MOE in the current study significantly increased the content of CAT and SOD in cardiac tissue, which are essential antioxidants for the protection of the heart. These effects could be due to MOE's relatively strong antioxidant activity (Al-Howiriny *et al*., 2009). The ability of MOE to counteract oxidative stress caused by DOXO was consistent with the findings of Ramadan *et al*. (2013) who reported that the MOE scavenged free radicals and blocked lipid peroxidation that is primarily due to MOE antioxidant properties. This work first revealed the inhibitory effect of MOE on lipid peroxidation and inflammatory cytokines, second, it normalized the production of SOD and CAT that could play an important role in the detoxification of peroxides and dangerous radicals against the DOXO.

Conclusion

The present results showed that MOE has good protection against DOXO's toxic effects. Therefore, MOE in combination with chemotherapy regarded as a potential candidate for reducing DOXO cardiotoxicity.

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