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Hawthorn Leaves Extract Suppress the Cardiotoxicity-induced by Doxorubicin in Rats: Mechanistic Study

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ABSTRACT

Cardiovascular diseases from the primary death causes worldwide. Doxorubicin (DOXO) consider one from the most widely and potent anticancer drugs. Free radicals are responsible for cardiotoxicity induced by DOXO. Hawthorn leaf has potent antioxidant, anti-cardiac remodeling, vasodilating, anti-inflammatory and anti-reperfusion/ischemia injury. The current work was aimed to investigate the possible action of hawthorn leaves methanolic extract (HLME) on the damage effects of DOXO in heart tissue. Adult male rats (n=40) were equally divided into 4 groups; Control (Con), DOXO, HLME and HLME+ DOXO groups. The HLME (400 mg/kg) was administrated for 3 weeks before intraperitoneal (i.p) injection with DOXO (20 mg /kg, single dose). Serum cardiac function enzymes, cardiac antioxidant biomarkers and serum inflammatory biomarkers were determined. As well as the cardiac muscle in all groups were histopathologically examined. Pre-treatment with HLME significantly lowered the elevated serum cardiac function activities and inflammatory cytokine biomarkers, as well as ameliorated cardiac antioxidant biomarkers via decreasing oxidative stress and increasing antioxidant status. The histopathological examination of cardiac muscle tissue confirmed these results. Therefore, the HLME has cardio protection effect against DOXO induced cardiotoxicity in rats, this effect could be explained via antioxidant and anti-inflammatory properties.

Keywords: *Hawthorn, Doxorubicin, Cardiotoxicity, Rats*

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INTRODUCTION

The first liposomal encapsulated anticancer drug was Doxorubicin hydrochloride liposomal injection. It has activity against a number of malignancies including solid tumors, transplantable leukemias, and lymphomas [1]. The DOXO has sever adverse effect such as cardiomyopathy, this disease is known to have a significant impact on the quality of life for patients who have survived cancer, particularly kids [2]. Several molecular processes is used to know the acute and chronic pathogenesis effect of DOXO that caused cardiotoxicity including oxidative stress, metabolism of iron, deregulation of Ca2+ homeostasis, changes in sarcomere composition, modulation of gene expression, and apoptosis.[3].

Different strategies including adrenergic receptor and iron-chelating antioxidants have been developed to protect the heart during cancer treatment. [4] However, the use of these drugs is limited due to their side effects, as well as the loss of useful heart effects years after the end of the treatment [5]. Therefore, the creation of other therapies was a significant challenge.

Several medicinal plants have successfully prevented DOXO-related cardiotoxicity exploring more plant-derived natural compounds that prevent DOXO cardiotoxicity and enhance its chemotherapeutic efficacy [6-8].

Crataegus, monogyna (Hawthorn) play an important role for folk medicine. Its flower buds, leaves and flowers are used to treat some diseases such as insomnia, irritability, loss of memory and confusion [9]. Hawthorn is used for cardiotonic, coronary vasodilatonic and hypotensive treatments [10]. The antioxidant property of hawthorn as one of the medicinal plants can be linked to its content of polyphenolic compounds including flavonoids [11]. The hawthorn contains bioactive compounds such as proanthocyanidins, phenolic acids, the essential oils, aromatic amines, and flavonoids (quercetin, hyperin, rutin, apigenin and spirein) [12]. Therefore, the current research aims to assess the effect of HLME in suppression the cardiotoxicity-induced by DOXO in rats.

MATERIAL AND METHODS

Drug and chemicals

Doxorubicin (Adriamcyin®, 50 mg Adr/25 ml) DOXO, EBEWE, Pharma Company, Austria, was bought from Sigma Co. (Aldrich Inc). All chemical with high grade were bought from Sigma (Aldrich chemical Co.) St. Louis, MO.

Preparation of Hawthorn leaves extraction

Hawthorn (*Crataegus* spp.) leaves *Rosacease* L. was purchased from the organic store Abazeer, Jeddah, KSA. The leaves of hawthorn was authenticated and identification in faculty of Pharmacy, KAU. KSA. Dried powdered of hawthorn leaves (500 g) was soaked in methanol 70 % (1 L) and mixed by magnetic stirrer (100 rpm) on a shaker for 48 h at 25°C . The resulting extract was then concentrated by rotary evaporator under vacuum at 37 °C after filtration then completely dried by freeze dried. Hawthorn leaves methanolic extract (HLME) was stored at -20°C until used for preparation the required concentration [13]. The HLME was given to the rats orally *via* gastric tube at 400 mg /kg b.wt [14] for 3 weeks [15].

Experiment protocol

Male rats (n=40) (200-220 g) were bought from the animal unit of King Fahd Center, KAU. Rats were adhering under the rules of Canadian Ethical that approved from Biomedical Ethical Committee, KAU. Rats fed standard pellet diet with free water. They were left 7 days to acclimatize. The animals were divided into control (Con), HLME and cardiotoxic (cardio) model groups; Cont group (n=10) rats injected with normal saline, HLME group (n=10) which injected orally with HLME (400 mg/kg), while cardio group were subdivided into Cardio (DOXO) (n=10) and cardioprotective (n=10) model group (HLME + DOXO) which injected orally with HLME (400 mg/kg) for 21 days, then injected with DOXO. Cardiotoxicity was induced *via* i.p. injection with DOXO (20 mg /kg, single dose) [16].

Blood and Heart samples collection

Three days after DOXO injection, the rats fasted 12 hrs before anaesthetized, the blood and tissue samples were collected. Serum samples were isolated for biochemical tests. The cardiac tissue samples were processed for histological and biochemical examinations.

Serum biochemical measurements

Cardiac function enzymes (creatinine kinase (CK-MB) activity and lactate dehydrogenase (LDH) activity) were measured in serum using ELISA kits purchased from My Biosource, San Diego, USA according to the instructions of the manufacture.

Cardiac antioxidant markers

Superoxide dismutase (SOD) and glutathione reductase (GR) activities, as well as the malonaldehyde (MDA) and nitric oxide (NO) levels were measured in cardiac tissue homogenate using ELISA kits purchased from My Biosource, San Diego, USA according to the manufacturer's instructions.

Serum inflammation markers

Interleukin-1 beta (IL-1 β) and Interlukin-6 (IL-6) were measured in serum using ELISA kits purchased from My Biosource, San Diego, USA according to the instructions of the manufacture.

Histopathological examination

Cardiac tissue samples were processed and stained with haematoxylin and eosin (H&E) for histopathological studies [17].

Statistics

The data were represented as (mean ± SE). Statistics was analyzed by ANOVA (one-way analysis of variance) using SPSS ver. 24 ($p \leq 0.05$ consider significant).

RESULTS

Cardiac function enzymes

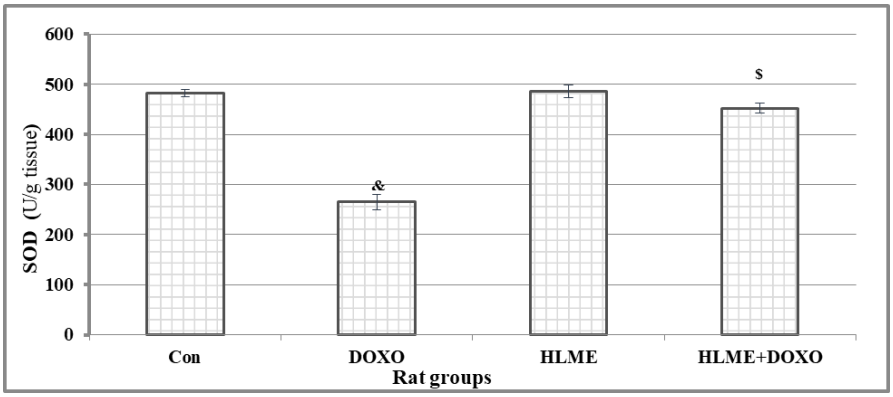
The The HLME (400 mg/kg) group revealed no significant difference on cardiac enzymes (CK-MB and LDH) compared with Con group. Injection of rats with DOXO showed significant ($p< 0.001$) elevation on cardiac function enzymes (CK-MB and LDH) compared with Con rats. While administration of HLME (400 mg/kg) pre-injection with DOXO exert significant decline on CK-MB and LDH activities compared with DOXO group Table 1.

TABLE 1: Effect of HLME on cardiac function enzymes CK-MB and LDH in DOXO- induced cardiotoxic in rats.

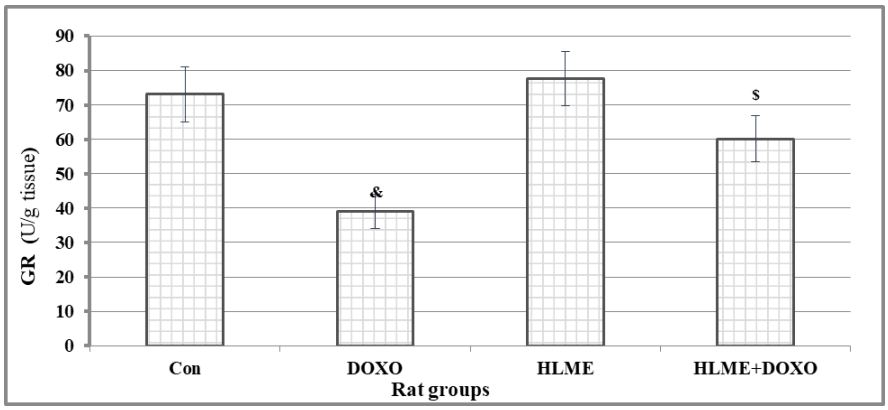
Animal groups	CK-MB	LDH
Con	251.21 ± 11.73	244.05 ± 10.60
DOXO	452.18 ± 11.54 &	534.17 ± 15.93 &
HLME	249.39 ± 9.75	243.49 ± 6.30
HLME+ DOXO	272.94 ±8.39 #	260.89 ±6.74 #

Cardiac antioxidant markers

The HLME (400 mg/kg) group showed improvement in cardiac antioxidant biomarkers, however there were no significant difference compared with Con group. Injection the rats with DOXO exerted exhaustion in cardiac antioxidant biomarkers, there were significant ($p< 0.001$) decline on cardiac enzymatic activities (SOD and GR) with significant ($p< 0.001$) elevation on non-enzymatic levels (MDA and NO) compared with Con rats. While administration of HLME (400 mg/kg) pre-injection with DOXO showed marked improvement on cardiac antioxidant biomarkers, there were significant ($p< 0.001$) elevation on cardiac enzymatic activities (SOD and GR) with significant ($p< 0.001$) decrease on non-enzymatic levels (MDA and NO) compared with DOXO group Figures 1-4.

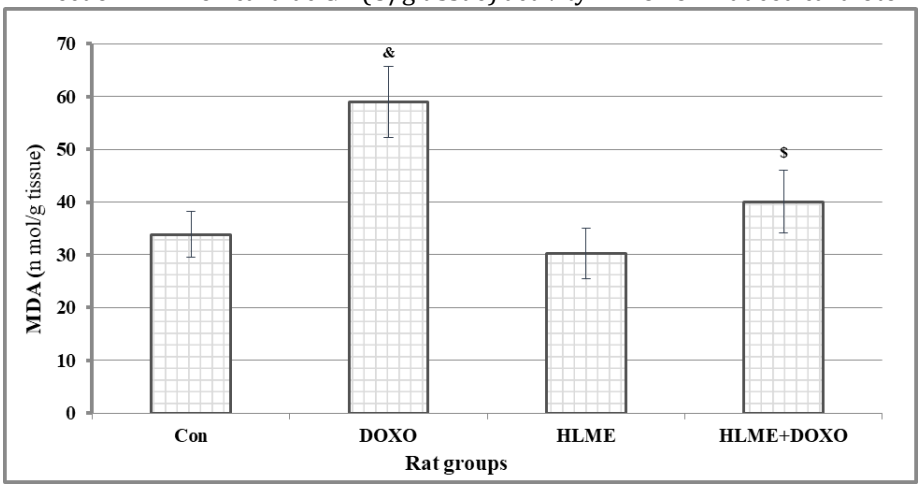


&significant difference versus Con group. $ Significant difference versus DOXO group. (n=10 rats/group)
Figure1. Effect of HLME on cardiac SOD (U/g tissue) activity in DOXO- induced cardiotoxic in rats.



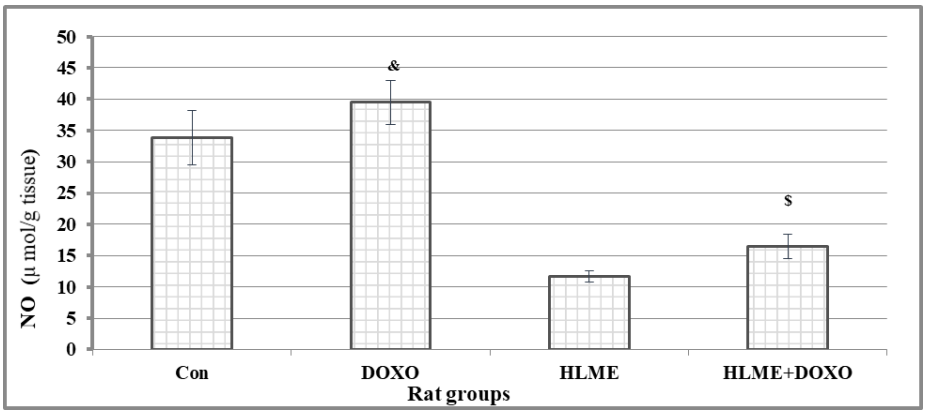
&significant difference versus Con group. $ Significant difference versus DOXO group. (n=10 rats/group)

Figure 2. Effect of HLME on cardiac GR (U/g tissue) activity in DOXO- induced cardiotoxic in rats.



&significant difference versus Con group. $ Significant difference versus DOXO group. (n=10 rats/group)

Figure 3. Effect of HLME on cardiac MDA (nmol/g tissue) activity in DOXO-induced cardiotoxic in rats.



&significant difference versus Con group. $ Significant difference versus DOXO group. (n=10 rats/group)

Figure 3. Effect of HLME on cardiac NO (µ mol/g tissue) activity in DOXO-induced cardiotoxic in rats.

Cardiac histopathological results

Cardiac muscle of Con and HLME groups (Figures 5 A and 5 E) showing normal cardiac myocytes. Cardiac muscle of DOXO group showing focal myocarditis with intermuscular leucocytic cells infiltration (Figure 5 B and Figure 5 C). As well as vacuolation of sarcoplasm of cardiac myocytes (Figure 5 D). Cardiac muscle of HLME +DOX group showing near normal structural of cardiac muscles (Figure 5 F).

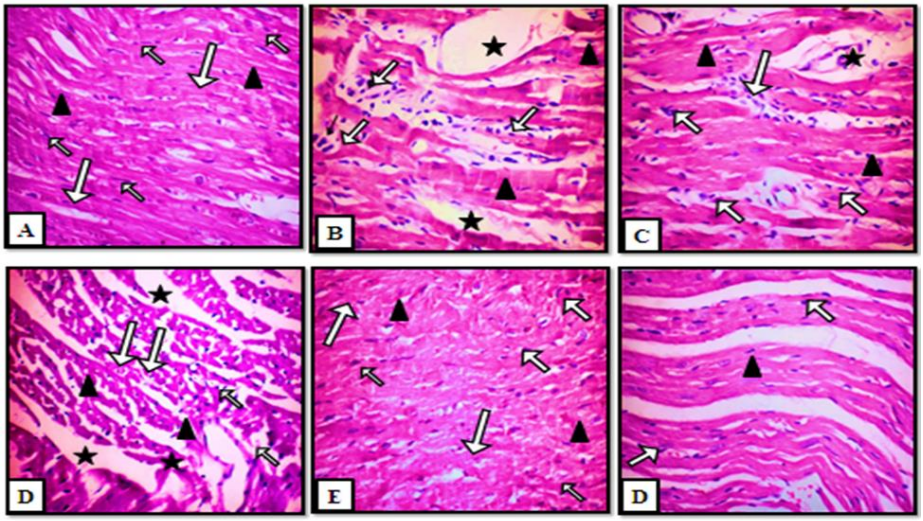


Figure 5. Effect of HLME on cardiac tissue in DOXO-induced cardiotoxic in rats (H&E x 400). Cardiac muscle of Con and HLME groups showing normal cardiac myocytes. Note oval central vesicular nuclei (small arrows), branching and striated muscle fiber (arrow head) and connective tissue cells with spindle shaped (large arrows) (Figures A and E). Cardiac muscle of DOXO group showing focal myocarditis with intermuscular leucocytic cells infiltration (small arrows), widely separated muscle cardiac (*), disorganized and degenerated cardiac fibers (arrow head) (Figure B and C). As well as vacuolation of sarcoplasm of cardiac myocytes (Figure D) (large arrows). Cardiac muscle of HLME+DOX group showing near normal structural appearance of cardiac muscles with organized cardiac fiber (arrows head) and oval central vesicular nuclei (arrows) (Figure F).

DISCUSSION

The DOXO is an outstanding antitumor medication to treat multiple kinds of leukemia, lymphomas and solid cancer [18]. The significant limiting complications is acute and chronic toxici-

ty.Symptoms of acute cardiotoxicity, such as arr hythmias, while chronic toxicity can advance into irreversible cardiomyopathy [19]. Doxorubicin has a risky dose-dependent effect in the development of cardiomyopathy in up to twenty five percent of patients and life-threatening heart failure in about 1-4%. Doxorubicin induced cardiotoxicity, which is linked with the cumulative dose of doxorubicin [20]. The DOXO constituents caused oxidative stress, which are the major rational for cardiotoxicity [21]. The current research was performed to explore the potential protective influences of HLME against cardiotoxicity caused by DOXO in experimental animals.

This study examined the impact of intraperitioneal injection with DOXO (20 mg /kg, single dose) on the cardiac function enzymes, cardiac antioxidant biomarkers and serum inflammatory biomarkers as well as histology of the rat's heart muscle compared to the control group. The data revealed that administration of DOXO had increased LDH, CK-MB enzymes activity, MDA, NO and serum inflammatory cytokines while there were significant decreases in SOD and GR enzymes. In addition, there were major histopathological changes in the heart muscle. Recently, most of the research results were in the same line with Atli *et al*. [22]. They reported that DOXO caused cardiotoxicity in rats. Higher concentrations of serum biomarker proteins (LDH and CK-MB) could indicate cardiotoxicity. These increases suggested a leakage of these enzymes from the cardiomyocytes which is backed by other studies [23-24]. The other

reason for cardiotoxicity caused by DOXO could be *via* free radical formation through two main mechanisms: a non-enzymatic pathway using iron and an enzymatic pathway using the respiratory chain of mitochondria [25- 26].

The DOXO-induced cardiotoxicity theory most widely involved the formation of free radicals and superoxides. In the free radical theory, the response was initiated by the loss of a doxorubicin electron that caused the development of radical doxorubicin semiquinone assisted by a de-

creased flavoenzyme like NADPH-cytochrome P450 reductase. This radical appears to be partially stable in the anoxic setting, but its unpaired electron is provided to oxygen under the normoxic condition leading to the creation of radicals of superoxide. The radical semiquinone created an iron complex resulting in the free radical doxorubicin-iron complex (Fe^{2+})[27-29]. This radical harm caused extremely toxic aldehydes like malondialdehyde (MAD) to be produced. These aldehydes could readily spread through the cell membrane into the cell and attach themselves to micromolecular objectives, thus acting as secondary cytotoxic messengers [30].

Interestingly, in this research that the co-administration or post-treatment of HLME improved oxidative damage and normalized DOXO's enzymatic and non-enzymatic defense operations, in addition to decreasing lipid peroxidation in the cardiac tissues of infected rats. Subsequently, the treated group's cardiac enzymes and other parameter values achieved close ordinary concentrations this may be due to HLME antioxidant scavenging ability. Hawthorn indicates a range of antioxidant-related polyphenols, flavonol glycosides, and C-glycosyl flavones. The HLME is modulating the toxic effect caused by DOXO [31-32]. The histopathological examination of cardiac tissue strongly supported these results. In the cardiac muscle, vacuolization are autophagic in nature and may rise due to the toxicity caused by DOXO which was depicted clearly in DOXO group. On the other hand the group of rats co-administered or post-treated with HLME, much less vacuolization was observed.

Hawthorn has been effectively used to treat cardiotoxicity through various activities, including vasodilatation of coronary and peripheral vessels by improving the endothelial release of nitric oxide and inhibition of angiotensin conversion enzyme (ACE), improving cardiac atrophy, and potentially by antiplatelet and anticoagulant impacts [33-35]. In relation to the immediate cholinergic receptor agonist, HLME also has a blocking effect of beta-adrenergic receptors [36]. Preliminary studies showed that the use of ACE and beta-blocking agents improves the cardiomyopathy caused by DOXO [37-38].

It could be concluded that HLME attenuates the cardiac damage induced by DOXO, it improved all biochemical parameters, as well as histopathological alterations *via* antioxidant and anti-inflammatory pathways.

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